

**84P2A9: A PROSTATE AND TESTIS SPECIFIC PROTEIN HIGHLY
EXPRESSED IN PROSTATE CANCER**

This application claims the benefit of United States provisional patent application number 60/178,560, filed January 26, 2000, the entire contents of which are incorporated herein by reference.

FIELD OF THE INVENTION

5 The invention described herein relates to a novel gene and its encoded protein, termed 84P2A9, and to diagnostic and therapeutic methods and compositions useful in the management of various cancers that express 84P2A9, particularly prostate cancers.

BACKGROUND OF THE INVENTION

10 Cancer is the second leading cause of human death next to coronary disease. Worldwide, millions of people die from cancer every year. In the United States alone, cancer causes the death of well over a half-million people annually, with some 1.4 million new cases diagnosed per year. While deaths from heart disease have been declining significantly, those resulting from cancer generally are on the rise. In the early part of the 15 next century, cancer is predicted to become the leading cause of death.

Worldwide, several cancers stand out as the leading killers. In particular, carcinomas of the lung, prostate, breast, colon, pancreas, and ovary represent the primary causes of cancer death. These and virtually all other carcinomas share a common lethal feature. With very few exceptions, metastatic disease from a carcinoma is fatal.

20 Moreover, even for those cancer patients who initially survive their primary cancers, common experience has shown that their lives are dramatically altered. Many cancer patients experience strong anxieties driven by the awareness of the potential for recurrence or treatment failure. Many cancer patients experience physical debilitations following treatment. Furthermore, many cancer patients experience a recurrence.

25 Worldwide, prostate cancer is the fourth most prevalent cancer in men. In North America and Northern Europe, it is by far the most common cancer in males and is the second leading cause of cancer death in men. In the United States alone, well over 40,000 men die annually of this disease - second only to lung cancer. Despite the

magnitude of these figures, there is still no effective treatment for metastatic prostate cancer. Surgical prostatectomy, radiation therapy, hormone ablation therapy, surgical castration and chemotherapy continue to be the main treatment modalities. Unfortunately, these treatments are ineffective for many and are often associated with
5 undesirable consequences.

On the diagnostic front, the lack of a prostate tumor marker that can accurately detect early-stage, localized tumors remains a significant limitation in the diagnosis and management of this disease. Although the serum prostate specific antigen (PSA) assay has been a very useful tool, however its specificity and general utility is widely regarded
10 as lacking in several important respects.

Progress in identifying additional specific markers for prostate cancer has been improved by the generation of prostate cancer xenografts that can recapitulate different stages of the disease in mice. The LAPC (Los Angeles Prostate Cancer) xenografts are prostate cancer xenografts that have survived passage in severe combined immune
15 deficient (SCID) mice and have exhibited the capacity to mimic the transition from androgen dependence to androgen independence (Klein et al., 1997, Nat. Med.3:402). More recently identified prostate cancer markers include PCTA-1 (Su et al., 1996, Proc. Natl. Acad. Sci. USA 93: 7252), prostate-specific membrane (PSM) antigen (Pinto et al., Clin Cancer Res 1996 Sep;2(9):1445-51), STEAP (Proc Natl Acad Sci U S A. 1999 Dec
20 7;96(25):14523-8) and prostate stem cell antigen (PSCA) (Reiter et al., 1998, Proc. Natl. Acad. Sci. USA 95: 1735).

While previously identified markers such as PSA, PSM, PCTA and PSCA have facilitated efforts to diagnose and treat prostate cancer, there is need for the identification of additional markers and therapeutic targets for prostate and related
25 cancers in order to further improve diagnosis and therapy.

SUMMARY OF THE INVENTION

The present invention relates to a novel, largely prostate and testis-related gene, designated 84P2A9, that is over-expressed in multiple cancers including prostate, testis,
30 kidney, brain, bone, skin, ovarian, breast, pancreas, colon, lymphocytic and lung cancers.

- Northern blot expression analysis of 84P2A9 gene expression in normal tissues shows a highly prostate and testis-related expression pattern in adult tissues. Analysis of 84P2A9 expression in normal prostate and prostate tumor xenografts shows over-expression in LAPC-4 and LAPC-9 prostate tumor xenografts, with the highest expression in LAPC-9.
- 5 The nucleotide (SEQ ID NO: 1) and amino acid (SEQ ID NO: 2) sequences of 84P2A9 are shown in FIG. 2. Portions of the 84P2A9 amino acid sequence show some homologies to ESTs in the dbEST database. The prostate and testis-related expression profile of 84P2A9 in normal adult tissues, combined with the over-expression observed in prostate tumor xenografts, shows that 84P2A9 is aberrantly over-expressed in at least
- 10 some cancers, and thus serves as a useful diagnostic and/or therapeutic target for cancers such as prostate, testis, kidney, brain, bone, skin, ovarian, breast, pancreas, colon, lymphocytic and lung cancers (see, e.g., FIGS. 4-8).

The invention provides polynucleotides corresponding or complementary to all or part of the 84P2A9 genes, mRNAs, and/or coding sequences, preferably in isolated form, including polynucleotides encoding 84P2A9 proteins and fragments of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more amino acids, DNA, RNA, DNA/RNA hybrids, and related molecules, polynucleotides or oligonucleotides complementary or having at least a 90% homology to the 84P2A9 genes or mRNA sequences or parts thereof, and polynucleotides or oligonucleotides that hybridize to the 84P2A9 genes, mRNAs, or to 20 84P2A9-encoding polynucleotides. Also provided are means for isolating cDNAs and the genes encoding 84P2A9. Recombinant DNA molecules containing 84P2A9 polynucleotides, cells transformed or transduced with such molecules, and host-vector systems for the expression of 84P2A9 gene products are also provided. The invention further provides antibodies that bind to 84P2A9 proteins and polypeptide fragments 25 thereof, including polyclonal and monoclonal antibodies, murine and other mammalian antibodies, chimeric antibodies, humanized and fully human antibodies, and antibodies labeled with a detectable marker.

The invention further provides methods for detecting the presence and status of 84P2A9 polynucleotides and proteins in various biological samples, as well as methods for 30 identifying cells that express 84P2A9. A typical embodiment of this invention provides

methods for monitoring 84P2A9 gene products in a tissue or hematology sample having or suspected of having some form of growth disregulation such as cancer.

The invention further provides various immunogenic or therapeutic compositions and strategies for treating cancers that express 84P2A9 such as prostate cancers, including
5 therapies aimed at inhibiting the transcription, translation, processing or function of 84P2A9 as well as cancer vaccines.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1. shows the 84P2A9 suppression subtractive hybridization (SSH) DNA
10 sequence of about 425 nucleotides in length (SEQ ID NO: 3). This sequence was identified in comparisons of cDNAs from various androgen dependent and androgen independent LAPC xenografts.

FIG. 2. shows the nucleotide (SEQ ID NO: 1) and amino acid (SEQ ID NO: 2)
15 sequences of 84P2A9. See Example 2, infra. The sequence surrounding the start ATG (AAC ATG G) (SEQ ID NO: 4) exhibits a Kozak sequence (A at position -3, and G at position +1). The start methionine with Kozak sequence is indicated in bold, the nuclear localization signals are boxed.

20 FIGS. 3A and 3B. show the amino acid sequence alignment of 84P2A9 (SEQ ID NO: 2) with KIAA1552 (SEQ ID NO: 5) and LUCA15 (SEQ ID NO: 6). FIG. 3A shows that the 84P2A9 protein sequence (bottom line) has some homology to the human brain protein KIAA1152 (39.5% identity over a 337 amino acid region, Score: 407.0; Gap frequency: 5.9%). FIG. 3B shows that the 84P2A9 protein sequence (bottom
25 line) contains a domain that is homologous to a portion of the LUCA15 tumor suppressor protein (64.3% identity over a 42 amino acid region, Score: 138.0; Gap frequency: 0.0%).

FIGS. 4A-4C. show the Northern blot analysis of the restricted 84P2A9
30 expression in various normal human tissues (using the 84P2A9 SSH fragment as a probe)

and LAPC xenografts. Two multiple tissue northern blots (Clontech) (FIGS. 4A and 4B) and a xenograft northern blot (FIG. 4C) were probed with the 84P2A9 SSH fragment. Lanes 1-8 in FIG. 4A consist of mRNA from heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas respectively. Lanes 1-8 in FIG. 4B consist of total RNA 5 from spleen, thymus, prostate, testis, ovary, small intestine, colon and leukocytes respectively. Lanes 1-5 in FIG. 4C consist of mRNA from prostate, LAPC-4 AD, LAPC-4 AI, LAPC-9 AD and LAPC-9 AI respectively. Size standards in kilobases (kb) are indicated on the side. Each lane contains 2 µg of mRNA for the normal tissues and 10 µg of total RNA for the xenograft tissues. The results show the expression of 10 84P2A9 in testis and prostate and the LAPC xenografts.

FIG. 5. shows the Northern blot analysis of 84P2A9 expression in prostate and multiple cancer cell lines. Lanes 1-56 show expression in LAPC-4 AD, LAPC-4 AI, LAPC-9 AD, LAPC-9 AI, LNCaP, PC-3, DU145, TsuPr1, LAPC-4 CL, HT1197, 15 SCaBER, UM-UC-3, TCCSUP, J82, 5637, 293T, RD-ES, PANC-1, BxPC-3, HPAC, Capan-1, SK-CO-1, CaCo-2, LoVo, T84, Colo-205, KCL 22, PFSK-1, T98G, SK-ES-1, HOS, U2-OS, RD-ES, CALU-1, A427, NCI-H82, NCI-H146, 769-P, A498, CAKI-1, SW839, BT20, CAMA-1, DU4475, MCF-7, MDA-MB-435s, NTERRA-2, NCCIT, TERA-1, TERA-2, A431, HeLa, OV-1063, PA-1, SW626 and CAOV-3 respectively. 20 High levels of 84P2A9 expression were detected in brain (PFSK-1, T98G), bone (HOS, U2-OS), lung (CALU-1, NCI-H82, NCI-H146), and kidney (769-P, A498, CAKI-1, SW839) cancer cell lines. Moderate expression levels were detected in several pancreatic (PANC-1, BxPC-3, HPAC, CAPAN-1), colon (SK-CO-1, CACO-2, LOVO, COLO-205), bone (SK-ES-1, RD-ES), breast (MCF-7, MDA-MB-435s) and testicular cancer 25 (NCCIT) cell lines.

FIG. 6. shows the Northern blot analysis of 84P2A9 expression in prostate cancer patient samples. Prostate cancer patient samples show expression of 84P2A9 in both the normal and the tumor part of the prostate tissues. Lanes 1-7 show Normal 30 prostate, Patient 1 normal adjacent tissue, Patient 1 Gleason 9 tumor, Patient 2 normal adjacent tissue, Patient 2 Gleason 7 tumor and Patient 3 Gleason 7 tumor respectively.

These results provide evidence that 84P2A9 is a very testis specific gene that is up-regulated in prostate cancer and potentially other cancers. Similar to the MAGE antigens, 84P2A9 may thus qualify as a cancer-testis antigen (Van den Eynde and Boon, Int J Clin Lab Res. 27:81-86, 1997).

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FIG. 7. shows RNA was isolated from kidney cancers (T) and their adjacent normal tissues (N) obtained from kidney cancer patients. Lanes 1-15 show 769-P- clear cell type; A498 - clear cell type; SW839 - clear cell type; Normal Kidney; Patient 1, N; Patient 1, tumor; Patient 2, N; Patient 2, tumor, clear cell type, grade III; Patient 3, N; Patient 3, tumor, clear cell type, grade II/IV; Patient 4, N; Patient 4, tumor, clear cell type, grade II/IV; Patient 5, N; Patient 5, tumor, clear cell type, grade II; and Patient 6, tumor, metastasis to chest wall respectively (N=normal adjacent tissue and CL=cell line). Northern analysis was performed using 10 μ g of total RNA for each sample. Expression of 84P2A9 was seen in all 6 tumor samples tested as well as in the three kidney cell lines, 10 769-P, A498 and SW839.

FIG. 8. shows RNA was isolated from colon cancers (T) and their adjacent normal tissues (N) obtained from colon cancer patients. Lanes 1-11 show Colo 205; LoVo; T84; Caco-2; Patient 1, N; Patient 1, tumor, grade 2, T3N1Mx (positive for lymph node metastasis); Patient 2, N; Patient 2, tumor, grade 1, T2N0Mx; Patient 3, N; Patient 20 3, tumor, grade 1, T2N1Mx (positive for lymph node metastasis); and Patient 4, tumor, grade 2, T3 N1 MX (positive for lymph node metastasis); respectively (N=normal adjacent tissue and CL=cell line). Northern analysis was performed using 10 μ g of total RNA for each sample. Expression of 84P2A9 was seen in all 4 tumor samples tested as well as in the 4 colon cancer cell lines Colo 205, LoVo, T84 and Caco-2.

FIG. 9. Shows expression of 84P2A9 assayed in a panel of human cancers (T) and their respective matched normal tissues (N) on RNA dot blots. Cancer cell lines from left to right are HeLa (cervical carcinoma), Daudi (Burkitt's lymphoma), K562 (CML), HL-60 (PML), G361 (melanoma), A549 (lung carcinoma), MOLT-4 (lymphoblastic leuk.), SW480 (colorectal carcinoma) and Raji (Burkitt's lymphoma).

84P2A9 expression was seen in kidney cancers, breast cancers, prostate cancers, lung cancers, stomach cancers, colon cancers, cervical cancers and rectum cancers. 84P2A9 was also found to be highly expressed in a panel of cancer cell lines, specially the MOLT-4 lymphoblastic leukemia and the A549 lung carcinoma cell lines. The expression 5 detected in normal adjacent tissues (isolated from diseased tissues) but not in normal tissues, isolated from healthy donors, can indicate that these tissues are not fully normal and that 84P2A9 can be expressed in early stage tumors.

FIG. 10 shows the expression of 84P2A9 in bladder cancer patient specimens. Expression of 84P2A9 was seen in 4 bladder cancer patient specimens tested and in 10 three bladder cell lines (CL), UM-UC-3 (lane 1), J82 (lane 2) and SCABER (lane 3). RNA was isolated from normal bladder (Nb), bladder tumors (T) and their adjacent normal tissues (N) obtained from 6 bladder cancer patients (P). Tumor from P1 is transitional carcinoma, grade 4; P2 is invasive squamous carcinoma; P3 is transitional carcinoma, grade 3; P4 is non-invasive papillary carcinoma, grade 1/3; P5 is papillary carcinoma, 15 grade 3/3; and P6 is transitional carcinoma, grade 3/2. Northern analysis was performed using 10 μ g of total RNA for each sample.

FIG. 11 shows the expression of 84P2A9 protein in 293T cells. 293T cells were transiently transfected with either pCDNA3.1 V5-HIS epitope tagged 84P2A9 plasmid or with empty control vector and harvested 2 days later. Cells were lysed in SDS-PAGE 20 sample buffer and lysates were separated on a 10-20% SDS-PAGE gel and then transferred to nitrocellulose. The blot was blocked in Tris-buffered saline (TBS)+ 2% non-fat milk and then probed with a 1:3,000 dilution of murine anti-V5 monoclonal Ab (Invitrogen) in TBS+0.15% Tween-20+ 1% milk. The blot was washed and then incubated with a 1:4,000 dilution of anti-mouse IgG-HRP conjugate secondary antibody. 25 Following washing, anti-V5 epitope immunoreactive bands were developed by enhanced chemiluminescence and visualized by exposure to autoradiographic film. Indicated by arrow is a specific anti-V5 immunoreactive band of approximately 87 Kd that corresponds to expression of the epitope-tagged 84P2A9 protein in the transfected cells.

DETAILED DESCRIPTION OF THE INVENTION

Unless otherwise defined, all terms of art, notations and other scientific terms or terminology used herein are intended to have the meanings commonly understood by those of skill in the art to which this invention pertains. In some cases, terms with
5 commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art. Many of the techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those
10 skilled in the art, such as, for example, the widely utilized molecular cloning methodologies described in Sambrook et al, Molecular Cloning: A Laboratory Manual 2nd edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. As appropriate, procedures involving the use of commercially available kits and reagents are generally carried out in accordance with manufacturer defined protocols and/or
15 parameters unless otherwise noted.

DEFINITIONS:

As used herein, the terms "advanced prostate cancer", "locally advanced prostate cancer", "advanced disease" and "locally advanced disease" mean prostate cancers that
20 have extended through the prostate capsule, and are meant to include stage C disease under the American Urological Association (AUA) system, stage C1 - C2 disease under the Whitmore-Jewett system, and stage T3 - T4 and N+ disease under the TNM (tumor, node, metastasis) system. In general, surgery is not recommended for patients with locally advanced disease, and these patients have substantially less favorable outcomes
25 compared to patients having clinically localized (organ-confined) prostate cancer. Locally advanced disease is clinically identified by palpable evidence of induration beyond the lateral border of the prostate, or asymmetry or induration above the prostate base. Locally advanced prostate cancer is presently diagnosed pathologically following radical prostatectomy if the tumor invades or penetrates the prostatic capsule, extends
30 into the surgical margin, or invades the seminal vesicles.

The term "antibody" is used in the broadest sense. Therefore an "antibody" can be naturally occurring or man made such as monoclonal antibodies produced by conventional hybridoma technology. Anti-84P2A9 antibodies comprise monoclonal and polyclonal antibodies as well as fragments containing the antigen binding domain and/or one or more 5 complementarity determining regions of these antibodies. As used herein, an antibody fragment is defined as at least a portion of the variable region of the immunoglobulin molecule that binds to its target, i.e., the antigen binding region. In one embodiment it specifically covers single anti-84P2A9 antibody (including agonist, antagonist and neutralizing antibodies) and anti-84P2A9 antibody compositions with polyepitopic 10 specificity. The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the antibodies comprising the population are identical except for possible naturally-occurring mutations that are present in minor amounts.

15 The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g. At²¹¹, I¹³¹, I¹²⁵, Y⁹⁰, Re¹⁸⁶, Re¹⁸⁸, Sm¹⁵³, Bi²¹², P³² and radioactive isotopes of Lu), chemotherapeutic agents, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof.

20 As used herein, the terms "hybridize", "hybridizing", "hybridizes" and the like, used in the context of polynucleotides, are meant to refer to conventional hybridization conditions, preferably such as hybridization in 50% formamide/6XSSC/0.1% SDS/100 µg/ml ssDNA, in which temperatures for hybridization are above 37 degrees C and temperatures for washing in 0.1XSSC/0.1% SDS are above 55 degrees C.

25 As used herein, a polynucleotide is said to be "isolated" when it is substantially separated from contaminant polynucleotides that correspond or are complementary to genes other than the 84P2A9 gene or that encode polypeptides other than 84P2A9 gene product or fragments thereof. A skilled artisan can readily employ nucleic acid isolation procedures to obtain an isolated 84P2A9 polynucleotide.

30 As used herein, a protein is said to be "isolated" when physical, mechanical or chemical methods are employed to remove the 84P2A9 protein from cellular constituents

that are normally associated with the protein. A skilled artisan can readily employ standard purification methods to obtain an isolated 84P2A9 protein.

The term "mammal" as used herein refers to any mammal classified as a mammal, including mice, rats, rabbits, dogs, cats, cows, horses and humans. In one preferred embodiment of the invention, the mammal is a mouse. In another preferred embodiment of the invention, the mammal is a human.

As used herein, the terms "metastatic prostate cancer" and "metastatic disease" mean prostate cancers that have spread to regional lymph nodes or to distant sites, and are meant to include stage D disease under the AUA system and stage TxNxM+ under the TNM system. As is the case with locally advanced prostate cancer, surgery is generally not indicated for patients with metastatic disease, and hormonal (androgen ablation) therapy is a preferred treatment modality. Patients with metastatic prostate cancer eventually develop an androgen-refractory state within 12 to 18 months of treatment initiation, and approximately half of these patients die within 6 months after developing androgen refractory status. The most common site for prostate cancer metastasis is bone. Prostate cancer bone metastases are often characteristically osteoblastic rather than osteolytic (i.e., resulting in net bone formation). Bone metastases are found most frequently in the spine, followed by the femur, pelvis, rib cage, skull and humerus. Other common sites for metastasis include lymph nodes, lung, liver and brain. Metastatic prostate cancer is typically diagnosed by open or laparoscopic pelvic lymphadenectomy, whole body radionuclide scans, skeletal radiography, and/or bone lesion biopsy.

"Moderately stringent conditions" are described by, identified but not limited to, those in Sambrook et al., Molecular Cloning: A Laboratory Manual, New York: Cold Spring Harbor Press, 1989, and include the use of washing solution and hybridization conditions (e.g., temperature, ionic strength and %SDS) less stringent than those described above. An example of moderately stringent conditions is overnight incubation at 37°C in a solution comprising: 20% formamide, 5 x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5 x Denhardt's solution, 10% dextran sulfate, and 20 mg/mL denatured sheared salmon sperm DNA, followed by washing the filters in 1 x SSC at about 37-50°C. The skilled artisan will recognize how to

adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

- As used herein "motif" as in biological motif of an 84P2A9-related protein, refers to any set of amino acids forming part of the primary sequence of a protein, either
- 5 contiguous or capable of being aligned to certain positions that are generally invariant or conserved, that is associated with a particular function or modification (e.g. that is phosphorylated, glycosylated or amidated).

As used herein, the term "polynucleotide" means a polymeric form of nucleotides of at least 10 bases or base pairs in length, either ribonucleotides or deoxynucleotides or

10 a modified form of either type of nucleotide, and is meant to include single and double stranded forms of DNA and/or RNA. In the art, this term if often used interchangeably with "oligonucleotide". As discussed herein, an polynucleotide can comprise a nucleotide sequence disclosed herein wherein thymidine (T) (as shown for example in SEQ ID NO: 1) can also be uracil (U). This description pertains to the differences

15 between the chemical structures of DNA and RNA, in particular the observation that one of the four major bases in RNA is uracil (U) instead of thymidine (T).

As used herein, the term "polypeptide" means a polymer of at least about 4, 5, 6, 7, or 8 amino acids. Throughout the specification, standard three letter or single letter designations for amino acids are used. In the art, this term if often used interchangeably

20 with "peptide".

"Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures.

25 Hybridization generally depends on the ability of denatured nucleic acid sequences to reanneal when complementary strands are present in an environment below their melting temperature. The higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature that can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions

30 more stringent, while lower temperatures less so. For additional details and explanation

of stringency of hybridization reactions, see Ausubel et al, Current Protocols in Molecular Biology, Wiley Interscience Publishers, (1995).

- "Stringent conditions" or "high stringency conditions", as defined herein, are identified by, but not limited to, those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 10 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (PH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 15 55°C.

A "transgenic animal" (e.g., a mouse or rat) is an animal having cells that contain a transgene, which transgene was introduced into the animal or an ancestor of the animal at a prenatal, e.g., an embryonic stage. A "transgene" is a DNA that is integrated into the genome of a cell from which a transgenic animal develops.

- 20 As used herein, the 84P2A9 gene and protein is meant to include the 84P2A9 genes and proteins specifically described herein and the genes and proteins corresponding to other 84P2A9 encoded proteins or peptides and structurally similar variants of the foregoing. Such other 84P2A9 peptides and variants will generally have coding sequences that are highly homologous to the 84P2A9 coding sequence, and 25 preferably share at least about 50% amino acid homology (using BLAST criteria) and preferably 50%, 60%, 70%, 80%, 90% or more nucleic acid homology, and at least about 60% amino acid homology (using BLAST criteria), more preferably sharing 70% or greater homology (using BLAST criteria).

The 84P2A9-related proteins of the invention include those specifically identified 30 herein, as well as allelic variants, conservative substitution variants and homologs that can be isolated/generated and characterized without undue experimentation following the

methods outlined herein or are readily available in the art. Fusion proteins that combine parts of different 84P2A9 proteins or fragments thereof, as well as fusion proteins of an 84P2A9 protein and a heterologous polypeptide are also included. Such 84P2A9 proteins are collectively referred to as the 84P2A9-related proteins, the proteins of the invention, or 5 84P2A9. As used herein, the term "84P2A9-related polypeptide" refers to a polypeptide fragment or an 84P2A9 protein sequence of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more amino acids

STRUCTURE AND EXPRESSION OF 84P2A9

- 10 As discussed in detail below, experiments with the LAPC-4 AD xenograft in male SCID mice have resulted in the identification of genes that are involved in the progression of androgen dependent (AD) prostate cancer to androgen independent (AI) cancer. Briefly, mice that harbored LAPC-4 AD xenografts were castrated when the tumors reached a size of 1 cm in diameter. The tumors regressed in size and temporarily
15 stopped producing the androgen dependent protein PSA. Seven to fourteen days post-castration, PSA levels were detectable again in the blood of the mice. Eventually such tumors develop an AI phenotype and start growing again in the castrated males. Tumors were harvested at different time points after castration to identify genes that are turned on or off during the transition to androgen independence.
- 20 Suppression subtractive hybridization (SSH) (Diatchenko et al., 1996, PNAS 93:6025) was then used to identify novel genes, such as those that are overexpressed in prostate cancer, by comparing cDNAs from various androgen dependent and androgen independent LAPC xenografts. This strategy resulted in the identification of novel genes exhibiting tissue and cancer specific expression. One of these genes, designated 84P2A9,
25 was identified from a subtraction where cDNA derived from an LAPC-4 AD tumor, 3 days post-castration, was subtracted from cDNA derived from an LAPC-4 AD tumor grown in an intact male. The SSH DNA sequence of about 425 bp (Fig. 1) is novel and exhibits homology only to expressed sequence tags (ESTs) in the dbEST database.
- 30 84P2A9, encodes a putative nuclear protein that exhibits prostate and testis-related expression. The initial characterization of 84P2A9 indicates that it is aberrantly expressed multiple cancers including prostate, testis, kidney, brain, bone, skin, ovarian,

breast, pancreas, colon, lymphocytic and lung cancers. The expression of 84P2A9 in prostate cancer provides evidence that this protein has a functional role in tumor progression. It is possible that 84P2A9 functions as a transcription factor involved in activating genes involved in tumorigenesis or repressing genes that block tumorigenesis.

5 As is further described in the Examples that follow, the 84P2A9 genes and proteins have been characterized using a number of analytical approaches. For example, analyses of nucleotide coding and amino acid sequences were conducted in order to identify potentially related molecules, as well as recognizable structural domains, topological features, and other elements within the 84P2A9 mRNA and protein
10 structures. Northern blot analyses of 84P2A9 mRNA expression were conducted in order to establish the range of normal and cancerous tissues expressing 84P2A9 message.

A full length 84P2A9 cDNA clone (clone 1) of 2345 base pairs (SEQ ID NO: 1) was cloned from an LAPC-4 AD cDNA library (Lambda ZAP Express, Stratagene) (Fig. 2). The cDNA encodes an open reading frame (ORF) of 504 amino acids (SEQ ID NO: 2). Sequence analysis revealed the presence of six potential nuclear localization signals and is predicted to be nuclear using the PSORT program (<http://psort.nibb.ac.jp:8800/form.html>). The protein sequence has some homology to a human brain protein KIAA1152 (SEQ ID NO: 5) (39.5% identity over a 337 amino acid region), and contains a domain that is homologous to the LUCA15 tumor suppressor protein (SEQ ID NO: 6) (64.3% identity over a 42 amino acid region)(GenBank Accession #P52756)(Fig. 3).

84P2A9 expression is prostate and testis-related in normal adult human tissues, but is also expressed in certain cancers, including prostate, testis, kidney, brain, bone, skin, ovarian, breast, pancreas, colon, lymphocytic and lung cancers. (see, e.g., FIGS. 4-25 8). Human prostate tumor xenografts originally derived from a patient with high grade metastatic prostate cancer express high levels of 84P2A9 (FIG. 4).

As disclosed herein, 84P2A9 exhibits specific properties that are analogous to those found in a family of genes whose polynucleotides, polypeptides, reactive cytotoxic T cells (CTL), helper T cells (HTL) and anti-polypeptide antibodies are used in well 30 known diagnostic assays directed to examining conditions associated with deregulated cell growth such as cancer, in particular prostate cancer (see, e.g., both its highly specific

pattern of tissue expression as well as its overexpression in prostate cancers as described for example in Example 3). The best known member of this class is PSA, the archetypal marker that has been used by medical practitioners for years to identify and monitor the presence of prostate cancer (see, e.g., Merrill et al., J. Urol. 163(2): 503-5120 (2000); 5 Polascik et al., J. Urol. Aug;162(2):293-306 (1999) and Fortier et al., J. Nat. Cancer Inst. 91(19): 1635-1640(1999)). A variety of other diagnostic markers are also used in this context including p53 and K-ras (see, e.g., Tulchinsky et al., Int J Mol Med 1999 Jul;4(1):99-102 and Minimoto et al., Cancer Detect Prev 2000;24(1):1-12). Therefore, this disclosure of the 84P2A9 polynucleotides and polypeptides (as well as the 84P2A9 10 polynucleotide probes and anti-84P2A9 antibodies used to identify the presence of these molecules) and their properties allows skilled artisans to utilize these molecules in methods that are analogous to those used, for example, in a variety of diagnostic assays directed to examining conditions associated with cancer.

Typical embodiments of diagnostic methods which utilize the 84P2A9 15 polynucleotides, polypeptides and antibodies described herein are analogous to those methods from well established diagnostic assays which employ PSA polynucleotides, polypeptides and antibodies. For example, just as PSA polynucleotides are used as probes (for example in Northern analysis, see, e.g., Sharief et al., Biochem. Mol. Biol. Int. 33(3):567-74(1994)) and primers (for example in PCR analysis, see, e.g., Okegawa et al., J. 20 Urol. 163(4): 1189-1190 (2000)) to observe the presence and/or the level of PSA mRNAs in methods of monitoring PSA overexpression or the metastasis of prostate cancers, the 84P2A9 polynucleotides described herein can be utilized in the same way to detect 84P2A9 overexpression or the metastasis of prostate and other cancers expressing this gene. Alternatively, just as PSA polypeptides are used to generate antibodies specific 25 for PSA which can then be used to observe the presence and/or the level of PSA proteins in methods of monitoring PSA protein overexpression (see, e.g., Stephan et al., Urology 55(4):560-3 (2000)) or the metastasis of prostate cells (see, e.g., Alanen et al., Pathol. Res. Pract. 192(3):233-7 (1996)), the 84P2A9 polypeptides described herein can be utilized to generate antibodies for use in detecting 84P2A9 overexpression or the 30 metastasis of prostate cells and cells of other cancers expressing this gene.

Specifically, because metastases involves the movement of cancer cells from an organ of origin (such as the testis or prostate gland etc.) to a different area of the body (such as a lymph node), assays which examine a biological sample for the presence of cells expressing 84P2A9 polynucleotides and/or polypeptides can be used to provide evidence of metastasis. For example, when a biological sample from tissue that does not normally contain 84P2A9 expressing cells (lymph node) is found to contain 84P2A9 expressing cells such as the 84P2A9 expression seen in LAPC4 and LAPC9, xenografts isolated from lymph node and bone metastasis, respectively, this finding is indicative of metastasis.

- 10 Alternatively 84P2A9 polynucleotides and/or polypeptides can be used to provide evidence of cancer, for example, when a cells in biological sample that do not normally express 84P2A9 or express 84P2A9 at a different level are found to express 84P2A9 or have an increased expression of 84P2A9 (see, e.g., the 84P2A9 expression in kidney, lung and colon cancer cells and in patient samples etc. shown in Figures 4-10).
- 15 In such assays, artisans may further wish to generate supplementary evidence of metastasis by testing the biological sample for the presence of a second tissue restricted marker (in addition to 84P2A9) such as PSA, PSCA etc. (see, e.g., Alonen et al., Pathol. Res. Pract. 192(3): 233-237 (1996)).

Just as PSA polynucleotide fragments and polynucleotide variants are employed by skilled artisans for use in methods of monitoring PSA, 84P2A9 polynucleotide fragments and polynucleotide variants are used in an analogous manner. In particular, typical PSA polynucleotides used in methods of monitoring PSA are probes or primers which consist of fragments of the PSA cDNA sequence. Illustrating this, primers used to PCR amplify a PSA polynucleotide must include less than the whole PSA sequence to function in the polymerase chain reaction. In the context of such PCR reactions, skilled artisans generally create a variety of different polynucleotide fragments that can be used as primers in order to amplify different portions of a polynucleotide of interest or to optimize amplification reactions (see, e.g., Caetano-Anolles, G. Biotechniques 25(3): 472-476, 478-480 (1998); Robertson et al., Methods Mol. Biol. 98:121-154 (1998)). An additional illustration of the use of such fragments is provided in Example 3, where an 84P2A9 polynucleotide fragment is used as a probe to show the overexpression of

84P2A9 mRNAs in cancer cells. In addition, in order to facilitate their use by medical practitioners, variant polynucleotide sequences are typically used as primers and probes for the corresponding mRNAs in PCR and Northern analyses (see, e.g., Sawai et al., *Fetal Diagn. Ther.* 1996 Nov-Dec;11(6):407-13 and *Current Protocols In Molecular Biology*,
5 Volume 2, Unit 2, Frederick M. Ausubel et al. eds., 1995)). Polynucleotide fragments and variants are typically useful in this context as long as they have the common attribute or characteristic of being capable of binding to a target polynucleotide sequence (e.g. the 84P2A9 polynucleotide shown in SEQ ID NO: 1) under conditions of high stringency.

Just as PSA polypeptide fragments and polypeptide variants are employed by
10 skilled artisans for use in methods of monitoring the PSA molecule, 84P2A9 polypeptide fragments and polypeptide variants can also be used in an analogous manner. In particular, typical PSA polypeptides used in methods of monitoring PSA are fragments of the PSA protein which contain an antibody epitope that can be recognized by an antibody or T cell that specifically binds to the PSA protein. This practice of using
15 polypeptide fragments or polypeptide variants to generate antibodies (such as anti-PSA antibodies or T cells) is typical in the art with a wide variety of systems such as fusion proteins being used by practitioners (see, e.g., *Current Protocols In Molecular Biology*, Volume 2, Unit 16, Frederick M. Ausubel et al. eds., 1995). In this context, each epitope(s) in a protein of interest functions to provide the architecture with which an
20 antibody or T cell is reactive. Typically, skilled artisans generally create a variety of different polypeptide fragments that can be used in order to generate antibodies specific for different portions of a polypeptide of interest (see, e.g., U.S. Patent No. 5,840,501 and U.S. Patent No. 5,939,533). For example it may be preferable to utilize a polypeptide comprising one of the 84P2A9 biological motifs discussed herein or
25 available in the art. Polypeptide fragments and variants or analogs are typically useful in this context as long as they comprise an epitope capable of generating an antibody or T cell specific for a target polypeptide sequence (e.g. the 84P2A9 polypeptide shown in SEQ ID NO: 2).

As shown herein, the 84P2A9 polynucleotides and polypeptides (as well as the
30 84P2A9 polynucleotide probes and anti-84P2A9 antibodies or T cells used to identify the presence of these molecules) exhibit specific properties that make them useful in

diagnosing cancers of the prostate. Diagnostic assays that measure the presence of 84P2A9 gene products, in order to evaluate the presence or onset of the particular disease conditions described herein such as prostate cancer are particularly useful in identifying patients for preventive measures or further monitoring, as has been done so 5 successfully with PSA. Moreover, these materials satisfy a need in the art for molecules having similar or complementary characteristics to PSA in situations where, for example, a definite diagnosis of metastasis of prostatic origin cannot be made on the basis of a testing for PSA alone (see, e.g., Alanan et al., Pathol. Res. Pract. 192(3): 233-237 (1996)), and consequently, materials such as 84P2A9 polynucleotides and polypeptides (as well as 10 the 84P2A9 polynucleotide probes and anti-84P2A9 antibodies used to identify the presence of these molecules) must be employed to confirm metastases of prostatic origin.

Finally, in addition to their use in diagnostic assays, the 84P2A9 polynucleotides disclosed herein have a number of other specific utilities such as their use in the identification of oncogenetic associated chromosomal abnormalities in 1q32.3. 15 Moreover, in addition to their use in diagnostic assays, the 84P2A9-related proteins and polynucleotides disclosed herein have other utilities such as their use in the forensic analysis of tissues of unknown origin (see, e.g., Takahama K Forensic Sci Int 1996 Jun 28;80(1-2): 63-9).

20 **84P2A9 POLYNUCLEOTIDES**

One aspect of the invention provides polynucleotides corresponding or complementary to all or part of an 84P2A9 gene, mRNA, and/or coding sequence, preferably in isolated form, including polynucleotides encoding an 84P2A9 protein and fragments thereof, DNA, RNA, DNA/RNA hybrid, and related molecules, 25 polynucleotides or oligonucleotides complementary to an 84P2A9 gene or mRNA sequence or a part thereof, and polynucleotides or oligonucleotides that hybridize to an 84P2A9 gene, mRNA, or to an 84P2A9 encoding polynucleotide (collectively, "84P2A9 polynucleotides").

One embodiment of an 84P2A9 polynucleotide is an 84P2A9 polynucleotide 30 having the sequence shown in SEQ ID NO: 1. An 84P2A9 polynucleotide can comprise

a polynucleotide having the nucleotide sequence of human 84P2A9 as shown in SEQ ID NO: 1, wherein T can also be U; a polynucleotide that encodes all or part of the 84P2A9 protein; a sequence complementary to the foregoing; or a polynucleotide fragment of any of the foregoing. Another embodiment comprises a polynucleotide having the sequence
5 as shown in SEQ ID NO: 1, from nucleotide residue number 163 through nucleotide residue number 1674, or from residue number 718 through residue number 1390, wherein T can also be U. Another embodiment comprises a polynucleotide encoding an 84P2A9 polypeptide whose sequence is encoded by the cDNA contained in the plasmid as deposited with American Type Culture Collection as Accession No. PTA-1151
10 Another embodiment comprises a polynucleotide that is capable of hybridizing under stringent hybridization conditions to the human 84P2A9 cDNA shown in SEQ ID NO: 1 or to a polynucleotide fragment thereof.

Typical embodiments of the invention disclosed herein include 84P2A9 polynucleotides encoding specific portions of the 84P2A9 mRNA sequence (and those
15 which are complementary to such sequences) such as those that encode the protein and fragments thereof, for example of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more contiguous amino acids. For example, representative embodiments of the invention disclosed herein include: polynucleotides encoding about amino acid position 1 to about amino acid 10 of the 84P2A9 protein shown in Fig. 2 (SEQ ID NO: 2), polynucleotides
20 encoding about amino acid 10 to about amino acid 20 of the 84P2A9 protein shown in Fig. 2, polynucleotides encoding about amino acid 20 to about amino acid 30 of the 84P2A9 protein shown in Fig. 2, polynucleotides encoding about amino acid 30 to about amino acid 40 of the 84P2A9 protein shown in Fig. 2, polynucleotides encoding about amino acid 40 to about amino acid 50 of the 84P2A9 protein shown in Fig. 2,
25 polynucleotides encoding about amino acid 50 to about amino acid 60 of the 84P2A9 protein shown in Fig. 2, polynucleotides encoding about amino acid 60 to about amino acid 70 of the 84P2A9 protein shown in Fig. 2, polynucleotides encoding about amino acid 70 to about amino acid 80 of the 84P2A9 protein shown in Fig. 2, polynucleotides encoding about amino acid 80 to about amino acid 90 of the 84P2A9 protein shown in
30 Fig. 2 and polynucleotides encoding about amino acid 90 to about amino acid 100 of the 84P2A9 protein shown in Fig. 2, etc. Following this scheme, polynucleotides (of at least

10 nucleic acids) encoding portions of the amino acid sequence of amino acids 100-504 of the 84P2A9 protein are typical embodiments of the invention.

Polynucleotides encoding larger portions of the 84P2A9 protein are also contemplated. For example polynucleotides encoding from about amino acid 1 (or 20 or 5 30 or 40 etc.) to about amino acid 20, (or 30, or 40 or 50 etc.) of the 84P2A9 protein shown in Fig. 2 can be generated by a variety of techniques well known in the art. An illustrative embodiment of such a polynucleotide consists of a polynucleotide having the sequence as shown in FIG. 2, from nucleotide residue number 718 through nucleotide residue number 1390.

10 Additional illustrative embodiments of the invention disclosed herein include 84P2A9 polynucleotide fragments encoding one or more of the biological motifs contained within the 84P2A9 protein sequence. In one embodiment, typical polynucleotide fragments of the invention can encode one or more of the nuclear localization sequences disclosed herein. In another embodiment, typical polynucleotide 15 fragments of the invention can encode one or more of the regions of 84P2A9 that exhibit homology to LUCA 15 and/or KIAA1152 and/or NY-Lu-12 lung cancer antigen (AF 042857), which exhibits Zinc finger and RNA binding motifs (see, e.g., Gure et al., Cancer Res. 58(5): 1034-1041 (1998). In another embodiment of the invention, typical polynucleotide fragments can encode one or more of the 84P2A9 N-glycosylation sites, 20 cAMP and cCMP-dependent protein kinase phosphorylation sites, casein kinase II phosphorylation sites or N-myristylation site and amidation sites as disclosed in greater detail in the text discussing the 84P2A9 protein and polypeptides herein. In yet another embodiment of the invention, typical polynucleotide fragments can encode sequences that are unique to one or more 84P2A9 alternative splicing variants, such as the splice 25 variant that generates the 4.5 KB transcript that is overexpressed in prostate cancers shown in FIG. 4.

The polynucleotides of the preceding paragraphs have a number of different specific uses. For example, because the human 84P2A9 gene maps to chromosome 30 1q32.3, polynucleotides encoding different regions of the 84P2A9 protein can be used to characterize cytogenetic abnormalities on chromosome 1, band q32 that have been identified as being associated with various cancers. In particular, a variety of

chromosomal abnormalities in 1q32 including translocations and deletions have been identified as frequent cytogenetic abnormalities in a number of different cancers (see, e.g., Bieche et al., Genes Chromosomes Cancer, 24(3): 255-263 (1999); Gorunova et al., Genes Chromosomes Cancer, 26(4): 312-321 (1999); Reid et al., Cancer Res. (22): 5415-5423 (1995)). Consequently, polynucleotides encoding specific regions of the 84P2A9 protein provide new tools that can be used to delineate with a greater precision than previously possible, the specific nature of the cytogenetic abnormalities in this region of chromosome 1 that can contribute to the malignant phenotype. In this context, these polynucleotides satisfy a need in the art for expanding the sensitivity of chromosomal screening in order to identify more subtle and less common chromosomal abnormalities (see, e.g., Evans et al., Am. J. Obstet. Gynecol 171(4): 1055-1057 (1994)).

Alternatively, as 84P2A9 is shown to be highly expressed in prostate cancers (Fig. 4), these polynucleotides can be used in methods assessing the status of 84P2A9 gene products in normal versus cancerous tissues. Typically, polynucleotides encoding specific regions of the 84P2A9 protein can be used to assess the presence of perturbations (such as deletions, insertions, point mutations, or alterations resulting in a loss of an antigen etc.) in specific regions (such regions containing a nuclear localization signal) of the 84P2A9 gene products. Exemplary assays include both RT-PCR assays as well as single-strand conformation polymorphism (SSCP) analysis (see, e.g., Marrogi et al., J. Cutan. Pathol. 26(8): 369-378 (1999), both of which utilize polynucleotides encoding specific regions of a protein to examine these regions within the protein.

Other specifically contemplated nucleic acid related embodiments of the invention disclosed herein are genomic DNA, cDNAs, ribozymes, and antisense molecules, as well as nucleic acid molecules based on an alternative backbone or including alternative bases, whether derived from natural sources or synthesized. For example, antisense molecules can be RNAs or other molecules, including peptide nucleic acids (PNAs) or non-nucleic acid molecules such as phosphorothioate derivatives, that specifically bind DNA or RNA in a base pair-dependent manner. A skilled artisan can readily obtain these classes of nucleic acid molecules using the 84P2A9 polynucleotides and polynucleotide sequences disclosed herein.

Antisense technology entails the administration of exogenous oligonucleotides that bind to a target polynucleotide located within the cells. The term "antisense" refers to the fact that such oligonucleotides are complementary to their intracellular targets, e.g., 84P2A9. See for example, Jack Cohen, OLIGODEOXYNUCLEOTIDES, 5 Antisense Inhibitors of Gene Expression, CRC Press, 1989; and Synthesis 1:1-5 (1988). The 84P2A9 antisense oligonucleotides of the present invention include derivatives such as S-oligonucleotides (phosphorothioate derivatives or S-oligos, see, Jack Cohen, *supra*), which exhibit enhanced cancer cell growth inhibitory action. S-oligos (nucleoside phosphorothioates) are isoelectronic analogs of an oligonucleotide (O-oligo) in which a 10 nonbridging oxygen atom of the phosphate group is replaced by a sulfur atom. The S-oligos of the present invention can be prepared by treatment of the corresponding O-oligos with 3H-1,2-benzodithiol-3-one-1,1-dioxide, which is a sulfur transfer reagent. See Iyer, R. P. et al, J. Org. Chem. 55:4693-4698 (1990); and Iyer, R. P. et al, J. Am. Chem. Soc. 112:1253-1254 (1990). Additional 84P2A9 antisense oligonucleotides of the 15 present invention include morpholino antisense oligonucleotides known in the art (see, e.g., Partridge et al., 1996, *Antisense & Nucleic Acid Drug Development* 6: 169-175).

The 84P2A9 antisense oligonucleotides of the present invention typically can be RNA or DNA that is complementary to and stably hybridizes with the first 100 N-terminal codons or last 100 C-terminal codons of the 84P2A9 genomic sequence or the 20 corresponding mRNA. Absolute complementarity is not required, although high degrees of complementarity are preferred. Use of an oligonucleotide complementary to this region allows for the selective hybridization to 84P2A9 mRNA and not to mRNA specifying other regulatory subunits of protein kinase. Preferably, the 84P2A9 antisense oligonucleotides of the present invention are a 15 to 30-mer fragment of the antisense 25 DNA molecule having a sequence that hybridizes to 84P2A9 mRNA. Optionally, 84P2A9 antisense oligonucleotide is a 30-mer oligonucleotide that is complementary to a region in the first 10 N-terminal codons or last 10 C-terminal codons of 84P2A9. Alternatively, the antisense molecules are modified to employ ribozymes in the inhibition 30 of 84P2A9 expression. L. A. Couture & D. T. Stinchcomb; *Trends Genet* 12: 510-515 (1996).

Further specific embodiments of this aspect of the invention include primers and primer pairs, which allow the specific amplification of the polynucleotides of the invention or of any specific parts thereof, and probes that selectively or specifically hybridize to nucleic acid molecules of the invention or to any part thereof. Probes can

5 be labeled with a detectable marker, such as, for example, a radioisotope, fluorescent compound, bioluminescent compound, a chemiluminescent compound, metal chelator or enzyme. Such probes and primers can be used to detect the presence of an 84P2A9 polynucleotide in a sample and as a means for detecting a cell expressing an 84P2A9 protein.

10 Examples of such probes include polypeptides comprising all or part of the human 84P2A9 cDNA sequences shown in FIG. 2. Examples of primer pairs capable of specifically amplifying 84P2A9 mRNAs are also described in the Examples that follow. As will be understood by the skilled artisan, a great many different primers and probes can be prepared based on the sequences provided herein and used effectively to amplify and/or 15 detect an 84P2A9 mRNA.

The 84P2A9 polynucleotides of the invention are useful for a variety of purposes, including but not limited to their use as probes and primers for the amplification and/or detection of the 84P2A9 gene(s), mRNA(s), or fragments thereof; as reagents for the diagnosis and/or prognosis of prostate cancer and other cancers; as coding sequences 20 capable of directing the expression of 84P2A9 polypeptides; as tools for modulating or inhibiting the expression of the 84P2A9 gene(s) and/or translation of the 84P2A9 transcript(s); and as therapeutic agents.

ISOLATION OF 84P2A9-ENCODING NUCLEIC ACID MOLECULES

25 The 84P2A9 cDNA sequences described herein enable the isolation of other polynucleotides encoding 84P2A9 gene product(s), as well as the isolation of polynucleotides encoding 84P2A9 gene product homologs, alternatively spliced isoforms, allelic variants, and mutant forms of the 84P2A9 gene product. Various molecular cloning methods that can be employed to isolate full length cDNAs encoding an 84P2A9 gene are 30 well known (See, for example, Sambrook, J. et al., Molecular Cloning: A Laboratory

Manual, 2d edition., Cold Spring Harbor Press, New York, 1989; Current Protocols in Molecular Biology. Ausubel et al., Eds., Wiley and Sons, 1995). For example, lambda phage cloning methodologies can be conveniently employed, using commercially available cloning systems (e.g., Lambda ZAP Express, Stratagene). Phage clones containing 84P2A9 gene cDNAs can be identified by probing with a labeled 84P2A9 cDNA or a fragment thereof. For example, in one embodiment, the 84P2A9 cDNA (FIG. 2) or a portion thereof can be synthesized and used as a probe to retrieve overlapping and full length cDNAs corresponding to an 84P2A9 gene. The 84P2A9 gene itself can be isolated by screening genomic DNA libraries, bacterial artificial chromosome libraries (BACs), yeast artificial chromosome libraries (YACs), and the like, with 84P2A9 DNA probes or primers.

RECOMBINANT DNA MOLECULES AND HOST-VECTOR SYSTEMS

The invention also provides recombinant DNA or RNA molecules containing an 84P2A9 polynucleotide or a fragment or analog or homologue thereof, including but not limited to phages, plasmids, phagemids, cosmids, YACs, BACs, as well as various viral and non-viral vectors well known in the art, and cells transformed or transfected with such recombinant DNA or RNA molecules. As used herein, a recombinant DNA or RNA molecule is a DNA or RNA molecule that has been subjected to molecular manipulation *in vitro*. Methods for generating such molecules are well known (see, for example, Sambrook et al, 1989, *supra*).

The invention further provides a host-vector system comprising a recombinant DNA molecule containing an 84P2A9 polynucleotide or fragment or analog or homologue thereof within a suitable prokaryotic or eukaryotic host cell. Examples of suitable eukaryotic host cells include a yeast cell, a plant cell, or an animal cell, such as a mammalian cell or an insect cell (e.g., a baculovirus-infectible cell such as an SF9 or HighFive cell). Examples of suitable mammalian cells include various prostate cancer cell lines such as DU145 and TsuPr1, other transfectable or transducible prostate cancer cell lines, as well as a number of mammalian cells routinely used for the expression of recombinant proteins (e.g., COS, CHO, 293, 293T cells). More particularly, a polynucleotide comprising the coding sequence of 84P2A9 or a fragment or analog or

homolog thereof can be used to generate 84P2A9 proteins or fragments thereof using any number of host-vector systems routinely used and widely known in the art.

A wide range of host-vector systems suitable for the expression of 84P2A9 proteins or fragments thereof are available, see for example, Sambrook et al., 1989, *supra*; Current Protocols in Molecular Biology, 1995, *supra*). Preferred vectors for mammalian expression include but are not limited to pcDNA 3.1 myc-His-tag (Invitrogen) and the retroviral vector pSRαtkneo (Muller et al., 1991, MCB 11:1785). Using these expression vectors, 84P2A9 may be preferably expressed in several prostate cancer and non-prostate cell lines, including for example 293, 293T, rat-1, NIH 3T3 and TsuPr1. The host-vector systems of the invention are useful for the production of an 84P2A9 protein or fragment thereof. Such host-vector systems can be employed to study the functional properties of 84P2A9 and 84P2A9 mutations or analogs.

Recombinant human 84P2A9 protein or an analog or homolog or fragment thereof can be produced by mammalian cells transfected with a construct encoding 84P2A9. In an illustrative embodiment described in the Examples, 293T cells can be transfected with an expression plasmid encoding 84P2A9 or fragment or analog or homolog thereof, the 84P2A9 or related protein is expressed in the 293T cells, and the recombinant 84P2A9 protein can be isolated using standard purification methods (e.g., affinity purification using anti-84P2A9 antibodies). In another embodiment, also described in the Examples herein, the 84P2A9 coding sequence is subcloned into the retroviral vector pSRαMSVtkneo and used to infect various mammalian cell lines, such as NIH 3T3, TsuPr1, 293 and rat-1 in order to establish 84P2A9 expressing cell lines. Various other expression systems well known in the art can also be employed. Expression constructs encoding a leader peptide joined in frame to the 84P2A9 coding sequence can be used for the generation of a secreted form of recombinant 84P2A9 protein.

Proteins encoded by the 84P2A9 genes, or by analogs or homologs or fragments thereof, will have a variety of uses, including but not limited to generating antibodies and in methods for identifying ligands and other agents and cellular constituents that bind to an 84P2A9 gene product. Antibodies raised against an 84P2A9 protein or fragment thereof can be useful in diagnostic and prognostic assays, and imaging methodologies in

the management of human cancers characterized by expression of 84P2A9 protein, including but not limited to cancers of the prostate and testis. Such antibodies can be expressed intracellularly and used in methods of treating patients with such cancers. Various immunological assays useful for the detection of 84P2A9 proteins are
5 contemplated, including but not limited to various types of radioimmunoassays, enzyme-linked immunosorbent assays (ELISA), enzyme-linked immunofluorescent assays (ELIFA), immunocytochemical methods, and the like. Such antibodies can be labeled and used as immunological imaging reagents capable of detecting 84P2A9 expressing cells (e.g., in radiosintigraphic imaging methods). 84P2A9 proteins can also be particularly useful in
10 generating cancer vaccines, as further described below.

84P2A9 POLYPEPTIDES

Another aspect of the present invention provides 84P2A9-related proteins and polypeptide fragments thereof. Specific embodiments of 84P2A9 proteins comprise a
15 polypeptide having all or part of the amino acid sequence of human 84P2A9 as shown in FIG. 2. Alternatively, embodiments of 84P2A9 proteins comprise variant polypeptides having alterations in the amino acid sequence of human 84P2A9 shown in FIG. 2.

In general, naturally occurring allelic variants of human 84P2A9 share a high degree
20 of structural identity and homology (e.g., 90% or more identity). Typically, allelic variants of the 84P2A9-related proteins contain conservative amino acid substitutions within the 84P2A9 sequences described herein or contain a substitution of an amino acid from a corresponding position in a homologue of 84P2A9. One class of 84P2A9 allelic variants are proteins that share a high degree of homology with at least a small region of a particular 84P2A9 amino acid sequence, but further contain a radical departure from the sequence,
25 such as a non-conservative substitution, truncation, insertion or frame shift. In comparisons of protein sequences, the terms, Similarity, identity, and Homology each have a distinct meaning. Moreover, Orthology and Paralogy are important concepts describing the relationship of members of a given protein family in one organism to the members of the same family in other organisms.

Conservative amino acid substitutions can frequently be made in a protein without altering either the conformation or the function of the protein. Such changes include substituting any of isoleucine (I), valine (V), and leucine (L) for any other of these hydrophobic amino acids; aspartic acid (D) for glutamic acid (E) and vice versa; 5 glutamine (Q) for asparagine (N) and vice versa; and serine (S) for threonine (T) and vice versa. Other substitutions can also be considered conservative, depending on the environment of the particular amino acid and its role in the three-dimensional structure of the protein. For example, glycine (G) and alanine (A) can frequently be interchangeable, as can alanine (A) and valine (V). Methionine (M), which is relatively 10 hydrophobic, can frequently be interchanged with leucine and isoleucine, and sometimes with valine. Lysine (K) and arginine (R) are frequently interchangeable in locations in which the significant feature of the amino acid residue is its charge and the differing pK's of these two amino acid residues are not significant. Still other changes can be considered "conservative" in particular environments (see, e.g. Table 2 herein; pages 13- 15 15 "Biochemistry" 2nd ED. Lubert Stryer ed (Stanford University); Henikoff et al., PNAS 1992 Vol 89 10915-10919; Lei et al., J Biol Chem 1995 May 19; 270(20):11882-6).

Embodiments of the invention disclosed herein include a wide variety of art accepted variants of 84P2A9 proteins such as polypeptides having amino acid insertions, deletions and substitutions. 84P2A9 variants can be made using methods known in the 20 art such as site-directed mutagenesis, alanine scanning, and PCR mutagenesis. Site-directed mutagenesis [Carter et al., *Nucl. Acids Res.*, 13:4331 (1986); Zoller et al., *Nucl. Acids Res.*, 10:6487 (1982)], cassette mutagenesis [Wells et al., *Gene*, 34:315 (1985)], restriction selection mutagenesis [Wells et al., *Philos. Trans. R. Soc. London Ser A*, 317:415 (1986)] or other known techniques can be performed on the cloned DNA to produce the 25 84P2A9 variant DNA.

Scanning amino acid analysis can also be employed to identify one or more amino acids along a contiguous sequence that is involved in a specific biological activity such as a protein-protein interaction. Among the preferred scanning amino acids are 30 relatively small, neutral amino acids. Such amino acids include alanine, glycine, serine, and cysteine. Alanine is typically a preferred scanning amino acid among this group because it eliminates the side-chain beyond the beta-carbon and is less likely to alter the

main-chain conformation of the variant. Alanine is also typically preferred because it is the most common amino acid. Further, it is frequently found in both buried and exposed positions [Creighton, *The Proteins*, (W.H. Freeman & Co., N.Y.); Chothia, J. Mol. Biol., 150:1 (1976)]. If alanine substitution does not yield adequate amounts of variant, 5 an isosteric amino acid can be used.

As defined herein, 84P2A9 variants, analogs or homologs, have the distinguishing attribute of having at least one epitope in common with an 84P2A9 protein having the amino acid sequence of SEQ ID NO: 2, such that an antibody or T cell that specifically binds to an 84P2A9 variant will also specifically bind to the 84P2A9 protein having the 10 amino acid sequence of SEQ ID NO: 2. A polypeptide ceases to be a variant of the protein shown in SEQ ID NO: 2 when it no longer contains an epitope capable of being recognized by an antibody or T cell that specifically binds to an 84P2A9 protein. Those skilled in the art understand that antibodies that recognize proteins bind to epitopes of varying size, and a grouping of the order of about four or five amino acids, contiguous or 15 not, is regarded as a typical number of amino acids in a minimal epitope. See, e.g., Nair et al., J. Immunol 2000 165(12): 6949-6955; Hebbes et al., Mol Immunol (1989) 26(9):865-73; Schwartz et al., J Immunol (1985) 135(4):2598-608. Another specific class of 84P2A9-related protein variants shares 90% or more identity with the amino acid sequence of SEQ ID NO: 2 or a fragment thereof. Another specific class of 84P2A9 20 protein variants or analogs comprise one or more of the 84P2A9 biological motifs described below or presently known in the art. Thus, encompassed by the present invention are analogs of 84P2A9 fragments (nucleic or amino acid) that altered functional (e.g. immunogenic) properties relative to the starting fragment. It is to be appreciated that motifs now or which become part of the art are to be applied to the 25 nucleic or amino acid sequences of FIG. 2.

As discussed herein, embodiments of the claimed invention include polypeptides containing less than the 504 amino acid sequence of the 84P2A9 protein shown in FIG. 2. For example, representative embodiments of the invention comprise peptides/proteins having any 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more contiguous 30 amino acids of the 84P2A9 protein shown in Fig. 2 (SEQ ID NO: 2). Moreover, representative embodiments of the invention disclosed herein include polypeptides

consisting of about amino acid 1 to about amino acid 10 of the 84P2A9 protein shown in Fig. 2, polypeptides consisting of about amino acid 10 to about amino acid 20 of the 84P2A9 protein shown in Fig. 2, polypeptides consisting of about amino acid 20 to about amino acid 30 of the 84P2A9 protein shown in Fig. 2, polypeptides consisting of
5 about amino acid 30 to about amino acid 40 of the 84P2A9 protein shown in Fig. 2, polypeptides consisting of about amino acid 40 to about amino acid 50 of the 84P2A9 protein shown in Fig. 2, polypeptides consisting of about amino acid 50 to about amino acid 60 of the 84P2A9 protein shown in Fig. 2, polypeptides consisting of about amino acid 60 to about amino acid 70 of the 84P2A9 protein shown in Fig. 2, polypeptides
10 consisting of about amino acid 70 to about amino acid 80 of the 84P2A9 protein shown in Fig. 2, polypeptides consisting of about amino acid 80 to about amino acid 90 of the 84P2A9 protein shown in Fig. 2 and polypeptides consisting of about amino acid 90 to about amino acid 100 of the 84P2A9 protein shown in Fig. 2, etc. throughout the entirety of the 84P2A9 sequence. Following this scheme, polypeptides consisting of
15 portions of the amino acid sequence of amino acids 100-504 of the 84P2A9 protein are typical embodiments of the invention. Polypeptides consisting of larger portions of the 84P2A9 protein are also contemplated. For example polypeptides consisting of about amino acid 1 (or 20 or 30 or 40 etc.) to about amino acid 20, (or 30, or 40 or 50 etc.) of the 84P2A9 protein shown in Fig. 2 can be generated by a variety of techniques well
20 known in the art. It is to be appreciated that the starting and stopping positions in this paragraph refer to the specified position as well as that position plus or minus 5 residues.

Additional illustrative embodiments of the invention disclosed herein include 84P2A9-related proteins containing the amino acid residues of one or more of the biological motifs contained within the 84P2A9-related protein sequence as shown in
25 Figure 2. In one embodiment, proteins of the invention comprise one or more of the 84P2A9 nuclear localization sequences such as RKRR at residues 42-45 of SEQ ID NO: 2, RKRR at residues 47-50 of SEQ ID NO: 2, KRRP at residues 101-104 of SEQ ID NO: 2, RRRRRK at residues 135-139 of SEQ ID NO: 2 and/or KKRK at residues 186-189 of SEQ ID NO: 2. In another embodiment, proteins of the invention comprise one
30 or more of the 84P2A9 N-glycosylation sites such as NRTL at residues 131-134 of SEQ ID NO: 2, NQTN at residues 212-215 of SEQ ID NO: 2 and/or NCSV at residues 394-

397 of SEQ ID NO: 2. In another embodiment, proteins of the invention comprise one or more of the regions of 84P2A9 that exhibit homology to LUCA 15 and/or KIAA1152. In another embodiment, proteins of the invention comprise one or more of the 84P2A9 cAMP and cGMP-dependent protein kinase phosphorylation sites such as

5 KRRS at residues 48-51 of SEQ ID NO: 2 and/or RRPS at residues 102-105 of SEQ ID NO: 2. In another embodiment, proteins of the invention comprise one or more of the 84P2A9 Protein Kinase C phosphorylation sites such as TLR at residues 133-135 of SEQ ID NO: 2, SNK at residues 152-154 of SEQ ID NO: 2, SDR at residues 171-173 of SEQ ID NO: 2, TNK at residues 214-216 of SEQ ID NO: 2, SRR at residues 313-315 of SEQ ID NO: 2, SSK at residues 328-330 of SEQ ID NO: 2 and/or SVR at residues 396-398 of SEQ ID NO: 2. In another embodiment, proteins of the invention comprise one or more of the 84P2A9 casein kinase II phosphorylation sites such as SALE at residues 10-13 of SEQ ID NO: 2, SSLE at residues 70-73 of SEQ ID NO: 2, SLEE at residues 71-74 of SEQ ID NO: 2, SDSD at residues 91-94 of SEQ ID NO: 2, TNKD at

10 residues 214-217 of SEQ ID NO: 2, SESD at residues 232-235 of SEQ ID NO: 2, SSTD at residues 240-243 of SEQ ID NO: 2, TNDE at residues 248-251 of SEQ ID NO: 2, TELD at residues 287-290 of SEQ ID NO: 2 and/or TEHD at residues 374-377 of SEQ ID NO: 2. In another embodiment, proteins of the invention comprise one or more of the N-myristoylation sites such as GSDSSL at residues 67-72 of SEQ ID NO: 2,

15 GLFTND at residues 245-250 of SEQ ID NO: 2, GGACGI at residues 269-274 of SEQ ID NO: 2, GGTPTS at residues 336-341 of SEQ ID NO: 2, GIPTSM at residues 337-342 of SEQ ID NO: 2, GSLCTG at residues 409-414 of SEQ ID NO: 2, GSGLGR at residues 459-464 of SEQ ID NO: 2 and/or GLGLGF at residues 481-486 of SEQ ID NO: 2. In another embodiment, proteins of the invention comprise one or more

20 amidation sites such as RGRK at residues 45-48 of SEQ ID NO: 2 and/or RGKR at residues 113-116 of SEQ ID NO: 2. An illustrative embodiment of such a polypeptide includes two or more amino acid sequences selected from the group consisting of KKRK, NQTN, NCSV, TNK, SRR, SSK, SVR, GLFTND, GGACGI, GGIPTS, GTPTSM and GSLCTG (as identified above in SEQ ID NO: 2). In a preferred

25 embodiment, the polypeptide comprises three or four or five or six or more amino acid sequences KKRK, NQTN, NCSV, TNK, SRR, SSK, SVR, GLFTND, GGACGI,

30

GGTPTS, GTPTSM and GSLCTG (as identified above in SEQ ID NO: 2).

In another embodiment, proteins of the invention comprise one or more of the immunoreactive epitopes identified by a process described herein such as such as those shown in Table 1. Processes for identifying peptides and analogues having affinities for

5 HLA molecules and which are correlated as immunogenic epitopes, are well known in the art. Also disclosed are principles for creating analogs of such epitopes in order to modulate immunogenicity. A variety of references are useful in the identification of such molecules. See, for example, WO 9733602 to Chestnut et al.; Sette, Immunogenetics 1999 50(3-4): 201-212; Sette et al., J. Immunol. 2001 166(2): 1389-1397; Alexander et al.,
10 Immunol. Res. 18(2): 79-92; Sidney et al., Hum. Immunol. 1997 58(1): 12-20; Kondo et al., Immunogenetics 1997 45(4): 249-258; Sidney et al., J. Immunol. 1996 157(8): 3480-90; and Falk et al., Nature 351: 290-6 (1991); Hunt et al., Science 255:1261-3 (1992); Parker et al., J. Immunol. 149:3580-7 (1992); Parker et al., J. Immunol. 152:163-75 (1994)); Kast et al., 1994 152(8): 3904-12; Borras-Cuesta et al., Hum. Immunol. 2000
15 61(3): 266-278; Alexander et al., J. Immunol. 2000 164(3); 164(3): 1625-1633; Alexander et al., PMID: 7895164, UI: 95202582; O'Sullivan et al., J. Immunol. 1991 147(8): 2663-2669; Alexander et al., Immunity 1994 1(9): 751-761 and Alexander et al., Immunol. Res. 1998 18(2): 79-92. The disclosures of these publications are hereby incorporated by reference herein in their entireties.

20 Related embodiments of the invention comprise polypeptides containing combinations of the different motifs discussed herein, where certain embodiments contain no insertions, deletions or substitutions either within the motifs or the intervening sequences of these polypeptides. In addition, embodiments which include a number of either N-terminal and/or C-terminal amino acid residues on either side of
25 these motifs may be desirable (to, for example, include a greater portion of the polypeptide architecture in which the motif is located). Typically the number of N-terminal and/or C-terminal amino acid residues on either side of a motif is between about 1 to about 100 amino acid residues, preferably 5 to about 50 amino acid residues.

30 In another embodiment of the invention, proteins of the invention comprise amino acid sequences that are unique to one or more 84P2A9 alternative splicing variants, such as the splice variant encoded by the 4.5 KB transcript that is overexpressed

in prostate cancers and shown in FIG. 4. The monitoring of alternative splice variants of 84P2A9 is useful because changes in the alternative splicing of proteins is suggested as one of the steps in a series of events that lead to the progression of cancers (see, e.g., Carstens et al., *Oncogene* 15(250): 3059-3065 (1997)). Consequently, monitoring of 5 alternative splice variants of 84P2A9 provides an additional means to evaluate syndromes associated with perturbations in 84P2A9 gene products such as cancers.

Polypeptides comprising one or more of the 84P2A9 motifs discussed herein are useful in elucidating the specific characteristics of a malignant phenotype in view of the observation that the 84P2A9 motifs discussed herein are associated with growth 10 disregulation and because 84P2A9 is overexpressed in cancers (FIG. 4). Thus, the presence in a protein of motifs related to these enzymes or molecules is relevant. For example, Casein kinase II, cAMP and cCMP-dependent protein kinase and Protein Kinase C for example are enzymes known to be associated with the development of the malignant phenotype (see, e.g., Chen et al., *Lab Invest.*, 78(2): 165-174 (1998); Gaiddon 15 et al., *Endocrinology* 136(10): 4331-4338 (1995); Hall et al., *Nucleic Acids Research* 24(6): 1119-1126 (1996); Peterziel et al., *Oncogene* 18(46): 6322-6329 (1999) and O'Brian, *Oncol. Rep.* 5(2): 305-309 (1998)). Moreover, both glycosylation and myristylation are protein modifications also associated with cancer and cancer progression (see, e.g., Dennis et al., *Biochim. Biophys. Acta* 1473(1):21-34 (1999); Raju et 20 al., *Exp. Cell Res.* 235(1): 145-154 (1997)). Amidation is another protein modification associated with cancer and cancer progression (see, e.g., Treston et al., *J. Natl. Cancer Inst. Monogr.* (13): 169-175 (1992)). In addition, nuclear localization sequences are believed to influence the malignant potential of a cell (see, e.g., Mirski et al., *Cancer Res.* 55(10): 2129-2134 (1995)).

25 The proteins of the invention have a number of different specific uses. As 84P2A9 is shown to be highly expressed in prostate cancers (Fig. 4), these peptides/proteins are used in methods assessing the status of 84P2A9 gene products in normal versus cancerous tissues and elucidating the malignant phenotype. Typically, polypeptides encoding specific regions of the 84P2A9 protein are used to assess the 30 presence of perturbations (such as deletions, insertions, point mutations etc.) in specific regions (such regions containing a nuclear localization signal) of the 84P2A9 gene

products. Exemplary assays utilize antibodies or T cells targeting 84P2A9-related proteins comprising the amino acid residues of one or more of the biological motifs contained within the 84P2A9 polypeptide sequence in order to evaluate the characteristics of this region in normal versus cancerous tissues. Alternatively, 84P2A9 5 polypeptides containing the amino acid residues of one or more of the biological motifs contained within the 84P2A9 proteins are used to screen for factors that interact with that region of 84P2A9.

As discussed herein, redundancy in the genetic code permits variation in 84P2A9 gene sequences. In particular, one skilled in the art will recognize specific codon 10 preferences by a specific host species, and can adapt the disclosed sequence as preferred for a desired host. For example, preferred analog codon sequences typically have rare codons (i.e., codons having a usage frequency of less than about 20% in known sequences of the desired host) replaced with higher frequency codons. Codon preferences for a specific species are calculated, for example, by utilizing codon usage 15 tables available on the INTERNET such as: <http://www.dna.affrc.go.jp/~nakamura/codon.html>. Nucleotide sequences that have been optimized for a particular host species by replacing any codons having a usage frequency of less than about 20% are referred to herein as "codon optimized sequences."

Additional sequence modifications are known to enhance protein expression in a 20 cellular host. These include elimination of sequences encoding spurious polyadenylation signals, exon/intron splice site signals, transposon-like repeats, and/or other such well-characterized sequences that are deleterious to gene expression. The GC content of the sequence is adjusted to levels average for a given cellular host, as calculated by reference to known genes expressed in the host cell. Where possible, the sequence is modified to 25 avoid predicted hairpin secondary mRNA structures. Other useful modifications include the addition of a translational initiation consensus sequence at the start of the open reading frame, as described in Kozak, *Mol. Cell Biol.*, 9:5073-5080 (1989). Skilled artisans understand that the general rule that eukaryotic ribosomes initiate translation exclusively at the 5' proximal AUG codon is abrogated only under rare conditions (see, e.g., Kozak 30 PNAS 92(7): 2662-2666, (1995) and Kozak NAR 15(20): 8125-8148 (1987)). Nucleotide sequences that have been optimized for expression in a given host species by elimination

of spurious polyadenylation sequences, elimination of exon/intron splicing signals, elimination of transposon-like repeats and/or optimization of GC content in addition to codon optimization are referred to herein as an “expression enhanced sequence.”

84P2A9 proteins are embodied in many forms, preferably in isolated form. A purified 84P2A9 protein molecule will be substantially free of other proteins or molecules that impair the binding of 84P2A9 to antibody or other ligand. The nature and degree of isolation and purification will depend on the intended use. Embodiments of an 84P2A9 protein include a purified 84P2A9 protein and a functional, soluble 84P2A9 protein. In one embodiment, a functional, soluble 84P2A9 protein or fragment thereof retains the ability to be bound by antibody, T cell or other ligand.

The invention also provides 84P2A9 proteins comprising biologically active fragments of the 84P2A9 amino acid sequence corresponding to part of the 84P2A9 amino acid sequence shown in FIG. 2. Such proteins of the invention exhibit properties of the 84P2A9 protein, such as the ability to elicit the generation of antibodies that specifically bind an epitope associated with the 84P2A9 protein; to be bound by such antibodies; to elicit the activation of HTL or CTL; and/or, to be recognized by HTL or CTL.

84P2A9-related proteins are generated using standard peptide synthesis technology or using chemical cleavage methods well known in the art. Alternatively, recombinant methods can be used to generate nucleic acid molecules that encode an 84P2A9-related protein. In one embodiment, the 84P2A9-encoding nucleic acid molecules described herein provide means for generating defined fragments of 84P2A9 proteins. 84P2A9 protein fragments/subsequences are particularly useful in generating and characterizing domain specific antibodies (e.g., antibodies recognizing an extracellular or intracellular epitope of an 84P2A9 protein), in identifying agents or cellular factors that bind to 84P2A9 or a particular structural domain thereof, and in various therapeutic contexts, including but not limited to cancer vaccines or methods of preparing such vaccines.

84P2A9 polypeptides containing particularly interesting structures can be predicted and/or identified using various analytical techniques well known in the art, including, for example, the methods of Chou-Fasman, Garnier-Robson, Kyte-Doolittle, Eisenberg, Karplus-Schultz or Jameson-Wolf analysis, or on the basis of immunogenicity. Fragments

containing such structures are particularly useful in generating subunit specific anti-84P2A9 antibodies, or T cells or in identifying cellular factors that bind to 84P2A9.

Illustrating this, the binding of peptides from 84P2A9 proteins to the human MHC class I molecule HLA-A2 were predicted. Specifically, the complete amino acid sequence of the 84P2A9 protein was entered into the HLA Peptide Motif Search algorithm found in the Bioinformatics and Molecular Analysis Section (BIMAS) Web site (<http://bimas.dcrt.nih.gov/>). The HLA Peptide Motif Search algorithm was developed by Dr. Ken Parker based on binding of specific peptide sequences in the groove of HLA Class I molecules and specifically HLA-A2 (see, e.g., Falk et al., *Nature* 351: 290-6 10 (1991); Hunt et al., *Science* 255:1261-3 (1992); Parker et al., *J. Immunol.* 149:3580-7 (1992); Parker et al., *J. Immunol.* 152:163-75 (1994)). This algorithm allows location and ranking of 8-mer, 9-mer, and 10-mer peptides from a complete protein sequence for predicted binding to HLA-A2 as well as numerous other HLA Class I molecules. Many HLA class I binding peptides are 8-, 9-, 10 or 11-mers. For example, for class I HLA-A2, the epitopes preferably contain a leucine (L) or methionine (M) at position 2 and a valine (V) or leucine (L) at the C-terminus (see, e.g., Parker et al., *J. Immunol.* 149:3580-7 15 (1992)). Selected results of 84P2A9 predicted binding peptides are shown in Table 1 below. It is to be appreciated that every epitope predicted by the DIMAS site, or specified by the HLA class I or class I motifs available in the art are to be applied (e.g., visually or by computer based methods, or appreciated by those of skill in the relevant 20 art) or which become part of the art are within the scope of the invention. In Table 1, the top 10 ranking candidates for each family member are shown along with their location, the amino acid sequence of each specific peptide, and an estimated binding score. The binding score corresponds to the estimated half-time of dissociation of complexes containing the peptide at 37°C at pH 6.5. Peptides with the highest binding 25 score (i.e. 63.04 for 84P2A9) are predicted to be the most tightly bound to HLA Class I on the cell surface for the greatest period of time and thus represent the best immunogenic targets for T-cell recognition. Actual binding of peptides to an HLA allele can be evaluated by stabilization of HLA expression on the antigen-processing defective 30 cell line T2 (see, e.g., Xue et al., *Prostate* 30:73-8 (1997) and Peshwa et al., *Prostate* 36:129-38 (1998)). Immunogenicity of specific peptides can be evaluated *in vitro* by

stimulation of CD8+ cytotoxic T lymphocytes (CTL) in the presence of antigen presenting cells such as dendritic cells.

In an embodiment described in the examples that follow, 84P2A9 can be conveniently expressed in cells (such as 293T cells) transfected with a commercially 5 available expression vector such as a CMV-driven expression vector encoding 84P2A9 with a C-terminal 6XHis and MYC tag (pcDNA3.1/mycHIS, Invitrogen or Tag5, GenHunter Corporation, Nashville TN). The Tag5 vector provides an IgGK secretion signal that can be used to facilitate the production of a secreted 84P2A9 protein in transfected cells. The secreted HIS-tagged 84P2A9 in the culture media can be purified, 10 e.g., using a nickel column using standard techniques.

Modifications of 84P2A9-related proteins such as covalent modifications are included within the scope of this invention. One type of covalent modification includes reacting targeted amino acid residues of an 84P2A9 polypeptide with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues of the 84P2A9. Another type of covalent modification of the 84P2A9 polypeptide included within the scope of this invention comprises altering the native glycosylation pattern of a protein of the invention. "Altering the native glycosylation pattern" is intended for purposes herein to mean deleting one or more carbohydrate moieties found in native sequence 84P2A9 (either by removing the underlying 15 glycosylation site or by deleting the glycosylation by chemical and/or enzymatic means), and/or adding one or more glycosylation sites that are not present in the native sequence 84P2A9. In addition, the phrase includes qualitative changes in the glycosylation of the native proteins, involving a change in the nature and proportions of the various carbohydrate moieties present. Another type of covalent modification of 84P2A9 20 comprises linking the 84P2A9 polypeptide to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol (PEG), polypropylene glycol, or polyoxalkylenes, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

The 84P2A9 of the present invention can also be modified in a way to form a 30 chimeric molecule comprising 84P2A9 fused to another, heterologous polypeptide or amino acid sequence. Such a chimeric molecule can be synthesized chemically or

recombinantly. A chimeric molecule can have a protein of the invention fused to another tumor-associated antigen or fragment thereof, or can comprise fusion of fragments of the 84P2A9 sequence (amino or nucleic acid) such that a molecule is created that is not, through its length, directly homologous to the amino or nucleic acid sequences respectively of FIG. 2 (SEQ ID NO: 2); such a chimeric molecule can comprise multiples of the same subsequence of 84P2A9. A chimeric molecule can comprise a fusion of an 84P2A9-related protein with a polyhistidine epitope tag, which provides an epitope to which immobilized nickel can selectively bind. The epitope tag is generally placed at the amino- or carboxyl- terminus of the 84P2A9. In an alternative embodiment, the chimeric molecule can comprise a fusion of an 84P2A9-related protein with an immunoglobulin or a particular region of an immunoglobulin. For a bivalent form of the chimeric molecule (also referred to as an "immunoadhesin"), such a fusion could be to the Fc region of an IgG molecule. The Ig fusions preferably include the substitution of a soluble (transmembrane domain deleted or inactivated) form of an 84P2A9 polypeptide in place of at least one variable region within an Ig molecule. In a particularly preferred embodiment, the immunoglobulin fusion includes the hinge, CH2 and CH3, or the hinge, CH1, CH2 and CH3 regions of an IgG1 molecule. For the production of immunoglobulin fusions see also US Patent No. 5,428,130 issued June 27, 1995.

20

84P2A9 ANTIBODIES

Another aspect of the invention provides antibodies that bind to 84P2A9-related proteins and polypeptides. Preferred antibodies specifically bind to an 84P2A9-related protein and will not bind (or will bind weakly) to non-84P2A9 proteins. In another embodiment, antibodies bind 84P2A9-related proteins as well as the homologs thereof.

25 84P2A9 antibodies of the invention are particularly useful in prostate cancer diagnostic and prognostic assays, and imaging methodologies. Similarly, such antibodies are useful in the treatment, diagnosis, and/or prognosis of other cancers, to the extent 84P2A9 is also expressed or overexpressed in other types of cancer. Moreover, intracellularly expressed antibodies (e.g., single chain antibodies) are therapeutically useful

in treating cancers in which the expression of 84P2A9 is involved, such as for example advanced and metastatic prostate cancers.

The invention also provides various immunological assays useful for the detection and quantification of 84P2A9 and mutant 84P2A9-related proteins. Such assays can 5 comprise one or more 84P2A9 antibodies capable of recognizing and binding an 84P2A9 or mutant 84P2A9 protein, as appropriate, and are performed within various immunological assay formats well known in the art, including but not limited to various types of radioimmunoassays, enzyme-linked immunosorbent assays (ELISA), enzyme-linked immunofluorescent assays (ELIFA), and the like.

10 Related immunological but non-antibody assays of the invention also comprise T cell immunogenicity assays (inhibitory or stimulatory) as well as major histocompatibility complex (MHC) binding assays. In addition, immunological imaging methods capable of detecting prostate cancer and other cancers expressing 84P2A9 are also provided by the invention, including but limited to radioscintigraphic imaging methods using labeled 15 84P2A9 antibodies. Such assays are clinically useful in the detection, monitoring, and prognosis of 84P2A9 expressing cancers such as prostate cancer.

84P2A9 antibodies can also be used in methods for purifying 84P2A9 and mutant 84P2A9 proteins and polypeptides and for isolating 84P2A9 homologues and related molecules. For example, in one embodiment, the method of purifying an 84P2A9 protein 20 comprises incubating an 84P2A9 antibody, which has been coupled to a solid matrix, with a lysate or other solution containing 84P2A9 under conditions that permit the 84P2A9 antibody to bind to 84P2A9; washing the solid matrix to eliminate impurities; and eluting the 84P2A9 from the coupled antibody. Other uses of the 84P2A9 antibodies of the invention include generating anti-idiotypic antibodies that mimic the 84P2A9 protein.

25 Various methods for the preparation of antibodies are well known in the art. For example, antibodies can be prepared by immunizing a suitable mammalian host using an 84P2A9-related protein, peptide, or fragment, in isolated or immunoconjugated form (Antibodies: A Laboratory Manual, CSH Press, Eds., Harlow, and Lane (1988); Harlow, Antibodies, Cold Spring Harbor Press, NY (1989)). In addition, fusion proteins of 84P2A9 30 can also be used, such as an 84P2A9 GST-fusion protein. In a particular embodiment, a GST fusion protein comprising all or most of the open reading frame amino acid sequence

of FIG. 2 is produced and used as an immunogen to generate appropriate antibodies. In another embodiment, an 84P2A9 peptide is synthesized and used as an immunogen.

In addition, naked DNA immunization techniques known in the art are used (with or without purified 84P2A9 protein or 84P2A9 expressing cells) to generate an immune response to the encoded immunogen (for review, see Donnelly et al., 1997, Ann. Rev. Immunol. 15: 617-648).

The amino acid sequence of 84P2A9 as shown in FIG. 2 can be used to select specific regions of the 84P2A9 protein for generating antibodies. For example, hydrophobicity and hydrophilicity analyses of the 84P2A9 amino acid sequence are used to identify hydrophilic regions in the 84P2A9 structure. Regions of the 84P2A9 protein that show immunogenic structure, as well as other regions and domains, can readily be identified using various other methods known in the art, such as Chou-Fasman, Garnier-Robson, Kyte-Doolittle, Eisenberg, Karplus-Schultz or Jameson-Wolf analysis. Thus, each region identified by any of these programs/methods is within the scope of the present invention. Methods for the generation of 84P2A9 antibodies are further illustrated by way of the examples provided herein.

Methods for preparing a protein or polypeptide for use as an immunogen and for preparing immunogenic conjugates of a protein with a carrier such as BSA, KLH, or other carrier proteins are well known in the art. In some circumstances, direct conjugation using, for example, carbodiimide reagents are used; in other instances linking reagents such as those supplied by Pierce Chemical Co., Rockford, IL, are effective. Administration of an 84P2A9 immunogen is conducted generally by injection over a suitable time period and with use of a suitable adjuvant, as is generally understood in the art. During the immunization schedule, titers of antibodies can be taken to determine adequacy of antibody formation.

84P2A9 monoclonal antibodies can be produced by various means well known in the art. For example, immortalized cell lines that secrete a desired monoclonal antibody are prepared using the standard hybridoma technology of Kohler and Milstein or modifications that immortalize producing B cells, as is generally known. The immortalized cell lines that secrete the desired antibodies are screened by immunoassay in which the antigen is an 84P2A9-related protein. When the appropriate immortalized cell culture secreting the

desired antibody is identified, the cells can be expanded and antibodies produced either from in vitro cultures or from ascites fluid.

The antibodies or fragments can also be produced, using current technology, by recombinant means. Regions that bind specifically to the desired regions of the 84P2A9 protein can also be produced in the context of chimeric or complementarity determining region (CDR) grafted antibodies of multiple species origin. Humanized or human 84P2A9 antibodies can also be produced and are preferred for use in therapeutic contexts. Methods for humanizing murine and other non-human antibodies, by substituting one or more of the non-human antibody CDRs for corresponding human antibody sequences, are well known (see for example, Jones et al., 1986, *Nature* 321: 522-525; Riechman et al., 1988, *Nature* 332: 323-327; Verhoeyen et al., 1988, *Science* 239: 1534-1536). See also, Carter et al., 1993, *Proc. Natl. Acad. Sci. USA* 89: 4285 and Sims et al., 1993, *J. Immunol.* 151: 2296. Methods for producing fully human monoclonal antibodies include phage display and transgenic methods (for review, see Vaughan et al., 1998, *Nature Biotechnology* 16: 535-539).

Fully human 84P2A9 monoclonal antibodies can be generated using cloning technologies employing large human Ig gene combinatorial libraries (i.e., phage display) (Griffiths and Hoogenboom, *Building an in vitro immune system: human antibodies from phage display libraries*. In: *Protein Engineering of Antibody Molecules for Prophylactic and Therapeutic Applications in Man*. Clark, M. (Ed.), Nottingham Academic, pp 45-64 (1993); Burton and Barbas, *Human Antibodies from combinatorial libraries*. *Id.*, pp 65-82). Fully human 84P2A9 monoclonal antibodies can also be produced using transgenic mice engineered to contain human immunoglobulin gene loci as described in PCT Patent Application WO98/24893, Kucherlapati and Jakobovits et al., published December 3, 1997 (see also, Jakobovits, 1998, *Exp. Opin. Invest. Drugs* 7(4): 607-614). This method avoids the in vitro manipulation required with phage display technology and efficiently produces high affinity authentic human antibodies.

Reactivity of 84P2A9 antibodies with an 84P2A9-related protein can be established by a number of well known means, including Western blot, 30 immunoprecipitation, ELISA, and FACS analyses using, as appropriate, 84P2A9-related proteins, peptides, 84P2A9-expressing cells or extracts thereof.

An 84P2A9 antibody or fragment thereof of the invention is labeled with a detectable marker or conjugated to a second molecule. Suitable detectable markers include, but are not limited to, a radioisotope, a fluorescent compound, a bioluminescent compound, chemiluminescent compound, a metal chelator or an enzyme. Further, bi-specific antibodies specific for two or more 84P2A9 epitopes are generated using methods generally known in the art. Homodimeric antibodies can also be generated by cross-linking techniques known in the art (e.g., Wolff et al., *Cancer Res.* 53: 2560-2565).

84P2A9 TRANSGENIC ANIMALS

- Nucleic acids that encode 84P2A9 or its modified forms can also be used to generate either transgenic animals or "knock out" animals which, in turn, are useful in the development and screening of therapeutically useful reagents. In accordance with established techniques, cDNA encoding 84P2A9 can be used to clone genomic DNA encoding 84P2A9 and the genomic sequences used to generate transgenic animals that contain cells that express DNA encoding 84P2A9. Methods for generating transgenic animals, particularly animals such as mice or rats, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009. Typically, particular cells would be targeted for 84P2A9 transgene incorporation with tissue-specific enhancers.
- Transgenic animals that include a copy of a transgene encoding 84P2A9 can be used to examine the effect of increased expression of DNA encoding 84P2A9. Such animals can be used as tester animals for reagents thought to confer protection from, for example, pathological conditions associated with its overexpression. In accordance with this facet of the invention, an animal is treated with a reagent and a reduced incidence of the pathological condition, compared to untreated animals bearing the transgene, would indicate a potential therapeutic intervention for the pathological condition.

Alternatively, non-human homologues of 84P2A9 can be used to construct an 84P2A9 "knock out" animal that has a defective or altered gene encoding 84P2A9 as a result of homologous recombination between the endogenous gene encoding 84P2A9 and altered genomic DNA encoding 84P2A9 introduced into an embryonic cell of the animal. For example, cDNA encoding 84P2A9 can be used to clone genomic DNA

encoding 84P2A9 in accordance with established techniques. A portion of the genomic DNA encoding 84P2A9 can be deleted or replaced with another gene, such as a gene encoding a selectable marker that can be used to monitor integration. Typically, several kilobases of unaltered flanking DNA (both at the 5' and 3' ends) are included in the
5 vector [see, e.g., Thomas and Capecchi, *Cell*, 51:503 (1987) for a description of homologous recombination vectors]. The vector is introduced into an embryonic stem cell line (e.g., by electroporation) and cells in which the introduced DNA has homologously recombined with the endogenous DNA are selected [see, e.g., Li et al.,
Cell, 69:915 (1992)]. The selected cells are then injected into a blastocyst of an animal
10 (e.g., a mouse or rat) to form aggregation chimeras [see, e.g., Bradley, in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E. J. Robertson, ed. (IRL, Oxford, 1987), pp. 113-152]. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term to create a "knock out" animal. Progeny harboring the homologously recombined DNA in their germ cells can be
15 identified by standard techniques and used to breed animals in which all cells of the animal contain the homologously recombined DNA. Knock out animals can be characterized for instance, for their ability to defend against certain pathological conditions and for their development of pathological conditions due to absence of the 84P2A9 polypeptide.

20

METHODS FOR THE DETECTION OF 84P2A9

Another aspect of the present invention relates to methods for detecting 84P2A9 polynucleotides and 84P2A9-related proteins and variants thereof, as well as methods for identifying a cell that expresses 84P2A9. 84P2A9 appears to be expressed in the LAPC
25 xenografts that are derived from lymph-node and bone metastasis of prostate cancer. The expression profile of 84P2A9 makes it a potential diagnostic marker for metastasized disease. In this context, the status of 84P2A9 gene products provide information useful for predicting a variety of factors including susceptibility to advanced stage disease, rate of progression, and/or tumor aggressiveness. As discussed in detail below, the status of
30 84P2A9 gene products in patient samples can be analyzed by a variety protocols that are

well known in the art including immunohistochemical analysis, the variety of Northern blotting techniques including in situ hybridization, RT-PCR analysis (for example on laser capture micro-dissected samples), Western blot analysis and tissue array analysis.

More particularly, the invention provides assays for the detection of 84P2A9 polynucleotides in a biological sample, such as serum, bone, prostate, and other tissues, urine, semen, cell preparations, and the like. Detectable 84P2A9 polynucleotides include, for example, an 84P2A9 gene or fragments thereof, 84P2A9 mRNA, alternative splice variant 84P2A9 mRNAs, and recombinant DNA or RNA molecules containing an 84P2A9 polynucleotide. A number of methods for amplifying and/or detecting the presence of 84P2A9 polynucleotides are well known in the art and can be employed in the practice of this aspect of the invention.

In one embodiment, a method for detecting an 84P2A9 mRNA in a biological sample comprises producing cDNA from the sample by reverse transcription using at least one primer; amplifying the cDNA so produced using an 84P2A9 polynucleotides as sense and antisense primers to amplify 84P2A9 cDNAs therein; and detecting the presence of the amplified 84P2A9 cDNA. Optionally, the sequence of the amplified 84P2A9 cDNA can be determined.

In another embodiment, a method of detecting an 84P2A9 gene in a biological sample comprises first isolating genomic DNA from the sample; amplifying the isolated genomic DNA using 84P2A9 polynucleotides as sense and antisense primers to amplify the 84P2A9 gene therein; and detecting the presence of the amplified 84P2A9 gene. Any number of appropriate sense and antisense probe combinations can be designed from the nucleotide sequences provided for the 84P2A9 (FIG. 2) and used for this purpose.

The invention also provides assays for detecting the presence of an 84P2A9 protein in a tissue or other biological sample such as serum, bone, prostate, and other tissues, urine, cell preparations, and the like. Methods for detecting an 84P2A9 protein are also well known and include, for example, immunoprecipitation, immunohistochemical analysis, Western Blot analysis, molecular binding assays, ELISA, ELIFA and the like. For example, in one embodiment, a method of detecting the presence of an 84P2A9 protein in a biological sample comprises first contacting the sample with an 84P2A9 antibody, an 84P2A9-reactive fragment thereof, or a recombinant protein containing an antigen

binding region of an 84P2A9 antibody; and then detecting the binding of 84P2A9 protein in the sample thereto.

- Methods for identifying a cell that expresses 84P2A9 are also provided. In one embodiment, an assay for identifying a cell that expresses an 84P2A9 gene comprises
- 5 detecting the presence of 84P2A9 mRNA in the cell. Methods for the detection of particular mRNAs in cells are well known and include, for example, hybridization assays using complementary DNA probes (such as *in situ* hybridization using labeled 84P2A9 riboprobes, Northern blot and related techniques) and various nucleic acid amplification assays (such as RT-PCR using complementary primers specific for 84P2A9, and other
- 10 amplification type detection methods, such as, for example, branched DNA, SISBA, TMA and the like). Alternatively, an assay for identifying a cell that expresses an 84P2A9 gene comprises detecting the presence of 84P2A9 protein in the cell or secreted by the cell. Various methods for the detection of proteins are well known in the art and are employed for the detection of 84P2A9 proteins and 84P2A9 expressing cells.
- 15 84P2A9 expression analysis is also useful as a tool for identifying and evaluating agents that modulate 84P2A9 gene expression. For example, 84P2A9 expression is significantly upregulated in prostate cancer, and is also expressed in other cancers including prostate, testis, kidney, brain, bone, skin, ovarian, breast, pancreas, colon, lymphocytic and lung cancers. Identification of a molecule or biological agent that could
- 20 inhibit 84P2A9 expression or over-expression in cancer cells is of therapeutic value. For example, such an agent can be identified by using a screen that quantifies 84P2A9 expression by RT-PCR, nucleic acid hybridization or antibody binding.

MONITORING THE STATUS OF 84P2A9 AND ITS PRODUCTS

- Assays that evaluate the status of the 84P2A9 gene and 84P2A9 gene products in
- 25 an individual can provide information on the growth or oncogenic potential of a biological sample from this individual. For example, because 84P2A9 mRNA is so highly expressed in prostate cancers (as well as the other cancer tissues shown for example in FIGS. 4-8) as compared to normal prostate tissue, assays that evaluate the relative levels of 84P2A9 mRNA transcripts or proteins in a biological sample can be used to diagnose a disease

associated with 84P2A9 disregulation such as cancer and can provide prognostic information useful in defining appropriate therapeutic options.

Because 84P2A9 is expressed, for example, in various prostate cancer xenograft tissues and cancer cell lines, and cancer patient samples, the expression status of 84P2A9 can provide information useful for determining information including the presence, stage and location of dysplastic, precancerous and cancerous cells, predicting susceptibility to various stages of disease, and/or for gauging tumor aggressiveness. Moreover, the expression profile makes it useful as an imaging reagent for metastasized disease. Consequently, an important aspect of the invention is directed to the various molecular prognostic and diagnostic methods for examining the status of 84P2A9 in biological samples such as those from individuals suffering from, or suspected of suffering from a pathology characterized by deregulated cellular growth such as cancer.

Oncogenesis is known to be a multistep process where cellular growth becomes progressively deregulated and cells progress from a normal physiological state to precancerous and then cancerous states (see, e.g., Alers et al., Lab Invest. 77(5): 437-438 (1997) and Isaacs et al., Cancer Surv. 23: 19-32 (1995)). In this context, examining a biological sample for evidence of deregulated cell growth (such as aberrant 84P2A9 expression in prostate cancers) can allow the early detection of such aberrant cellular physiology before a pathology such as cancer has progressed to a stage at which therapeutic options are more limited. In such examinations, the status of 84P2A9 in a biological sample of interest (such as one suspected of having deregulated cell growth) can be compared, for example, to the status of 84P2A9 in a corresponding normal sample (e.g. a sample from that individual (or alternatively another individual) that is not effected by a pathology, for example one not suspected of having deregulated cell growth). Alterations in the status of 84P2A9 in the biological sample of interest (as compared to the normal sample) provides evidence of deregulated cellular growth. In addition to using a biological sample that is not effected by a pathology as a normal sample, one can also use a predetermined normative value such as a predetermined normal level of mRNA expression (see, e.g., Grever et al., J. Comp. Neurol. 1996 Dec 9;376(2):306-14 and U.S. patent No. 5,837,501) to compare 84P2A9 in normal versus suspect samples.

The term "status" in this context is used according to its art accepted meaning and refers to the condition or state of a gene and its products. Typically, skilled artisans use a number of parameters to evaluate the condition or state of a gene and its products. These include, but are not limited to the location of expressed gene products (including the 5 location of 84P2A9 expressing cells) as well as the, level, and biological activity of expressed gene products (such as 84P2A9 mRNA polynucleotides and polypeptides). Alterations in the status of 84P2A9 can be evaluated by a wide variety of methodologies well known in the art, typically those discussed herein. Typically an alteration in the status of 84P2A9 comprises a change in the location of 84P2A9 and/or 84P2A9 10 expressing cells and/or an increase in 84P2A9 mRNA and/or protein expression.

As discussed in detail herein, in order to identify a condition or phenomenon associated with deregulated cell growth, the status of 84P2A9 in a biological sample is evaluated by a number of methods utilized by skilled artisans including, but not limited to genomic Southern analysis (to examine, for example perturbations in the 84P2A9 15 gene), Northern analysis and/or PCR analysis of 84P2A9 mRNA (to examine, for example alterations in the polynucleotide sequences or expression levels of 84P2A9 mRNAs), and Western and/or immunohistochemical analysis (to examine, for example alterations in polypeptide sequences, alterations in polypeptide localization within a sample, alterations in expression levels of 84P2A9 proteins and/or associations of 20 84P2A9 proteins with polypeptide binding partners). Detectable 84P2A9 polynucleotides include, for example, an 84P2A9 gene or fragments thereof, 84P2A9 mRNA, alternative splice variants 84P2A9 mRNAs, and recombinant DNA or RNA molecules containing an 84P2A9 polynucleotide.

The expression profile of 84P2A9 makes it a diagnostic marker for local and/or 25 metastasized disease. In particular, the status of 84P2A9 provides information useful for predicting susceptibility to particular disease stages, progression, and/or tumor aggressiveness. The invention provides methods and assays for determining 84P2A9 status and diagnosing cancers that express 84P2A9, such as cancers of the prostate, bladder, testis, ovaries, breast, pancreas, colon and lung. 84P2A9 status in patient samples can be 30 analyzed by a number of means well known in the art, including without limitation, immunohistochemical analysis, in situ hybridization, RT-PCR analysis on laser capture

micro-dissected samples, Western blot analysis of clinical samples and cell lines, and tissue array analysis. Typical protocols for evaluating the status of the 84P2A9 gene and gene products can be found, for example in Ausubel et al. eds, 1995, Current Protocols In Molecular Biology, Units 2 [Northern Blotting], 4 [Southern Blotting], 15

5 [Immunoblotting] and 18 [PCR Analysis].

As described above, the status of 84P2A9 in a biological sample can be examined by a number of well known procedures in the art. For example, the status of 84P2A9 in a biological sample taken from a specific location in the body can be examined by evaluating the sample for the presence or absence of 84P2A9 expressing cells (e.g. those

10 that express 84P2A9 mRNAs or proteins). This examination can provide evidence of deregulated cellular growth, for example, when 84P2A9 expressing cells are found in a biological sample that does not normally contain such cells (such as a lymph node). Such alterations in the status of 84P2A9 in a biological sample are often associated with deregulated cellular growth. Specifically, one indicator of deregulated cellular growth is

15 the metastases of cancer cells from an organ of origin (such as the testis or prostate gland) to a different area of the body (such as a lymph node). In this context, evidence of deregulated cellular growth is important for example because occult lymph node metastases can be detected in a substantial proportion of patients with prostate cancer, and such metastases are associated with known predictors of disease progression (see,

20 e.g., Murphy et al., Prostate 42(4): 315-317 (2000); Su et al., Semin. Surg. Oncol. 18(1): 17-28 (2000) and Freeman et al., J Urol 1995 Aug;154(2 Pt 1):474-8).

In one aspect, the invention provides methods for monitoring 84P2A9 gene products by determining the status of 84P2A9 gene products expressed by cells in a test tissue sample from an individual suspected of having a disease associated with

25 deregulated cell growth (such as hyperplasia or cancer) and then comparing the status so determined to the status of 84P2A9 gene products in a corresponding normal sample, the presence of aberrant 84P2A9 gene products in the test sample relative to the normal sample providing an indication of the presence of deregulated cell growth within the cells of the individual.

30 In another aspect, the invention provides assays useful in determining the presence of cancer in an individual, comprising detecting a significant increase in 84P2A9

mRNA or protein expression in a test cell or tissue sample relative to expression levels in the corresponding normal cell or tissue. The presence of 84P2A9 mRNA can, for example, be evaluated in tissue samples including but not limited to prostate, testis, kidney, brain, bone, skin, ovarian, breast, pancreas, colon, lymphocytic and lung tissues
5 (see, e.g., FIGS. 4-8). The presence of significant 84P2A9 expression in any of these tissues is useful to indicate the emergence, presence and/or severity of a cancer, since the corresponding normal tissues do not express 84P2A9 mRNA or express it at lower levels.

In a related embodiment, 84P2A9 status is determined at the protein level rather
10 than at the nucleic acid level. For example, such a method or assay comprises determining the level of 84P2A9 protein expressed by cells in a test tissue sample and comparing the level so determined to the level of 84P2A9 expressed in a corresponding normal sample. In one embodiment, the presence of 84P2A9 protein is evaluated, for example, using immunohistochemical methods. 84P2A9 antibodies or binding partners capable of
15 detecting 84P2A9 protein expression are used in a variety of assay formats well known in the art for this purpose.

In other related embodiments, one can evaluate the status 84P2A9 nucleotide and amino acid sequences in a biological sample in order to identify perturbations in the structure of these molecules such as insertions, deletions, substitutions and the like. Such
20 embodiments are useful because perturbations in the nucleotide and amino acid sequences are observed in a large number of proteins associated with a growth disregulated phenotype (see, e.g., Marrogi et al., 1999, J. Cutan. Pathol. 26(8):369-378). For example, a mutation is the sequence of 84P2A9 may be indicative of the presence or promotion of a tumor. Such assays can therefore have diagnostic and predictive value where a mutation in 84P2A9
25 indicates a potential loss of function or increase in tumor growth.

A wide variety of assays for observing perturbations in nucleotide and amino acid sequences are well known in the art. For example, the size and structure of nucleic acid or amino acid sequences of 84P2A9 gene products are observed by the Northern, Southern, Western, PCR and DNA sequencing protocols discussed herein. In addition, other
30 methods for observing perturbations in nucleotide and amino acid sequences such as single

strand conformation polymorphism analysis are well known in the art (see, e.g., U.S. Patent Nos. 5,382,510 and 5,952,170).

In another embodiment, one can examine the methylation status of the 84P2A9 gene in a biological sample. Aberrant demethylation and/or hypermethylation of CpG islands in gene 5' regulatory regions frequently occurs in immortalized and transformed cells and can result in altered expression of various genes. For example, promoter hypermethylation of the pi-class glutathione S-transferase (a protein expressed in normal prostate but not expressed in >90% of prostate carcinomas) appears to permanently silence transcription of this gene and is the most frequently detected genomic alteration in prostate carcinomas (De Marzo et al., Am. J. Pathol. 155(6): 1985-1992 (1999)). In addition, this alteration is present in at least 70% of cases of high-grade prostatic intraepithelial neoplasia (PIN) (Brooks et al, Cancer Epidemiol. Biomarkers Prev., 1998, 7:531-536). In another example, expression of the LAGE-I tumor specific gene (which is not expressed in normal prostate but is expressed in 25-50% of prostate cancers) is induced by deoxy-azacytidine in lymphoblastoid cells, suggesting that tumoral expression is due to demethylation (Lethe et al., Int. J. Cancer 76(6): 903-908 (1998)). In this context, a variety of assays for examining methylation status of a gene are well known in the art. For example, one can utilize, in Southern hybridization approaches, methylation-sensitive restriction enzymes which can not cleave sequences that contain methylated CpG sites, in order to assess the overall methylation status of CpG islands. In addition, MSP (methylation specific PCR) can rapidly profile the methylation status of all the CpG sites present in a CpG island of a given gene. This procedure involves initial modification of DNA by sodium bisulfite (which will convert all unmethylated cytosines to uracil) followed by amplification using primers specific for methylated versus unmethylated DNA. Protocols involving methylation interference can also be found for example in Current Protocols In Molecular Biology, Units 12, Frederick M. Ausubel et al. eds., 1995.

Gene amplification provides an additional method of assessing the status of 84P2A9, a locus that maps to 1q32.3, a region shown to be perturbed in a variety of cancers. Gene amplification is measured in a sample directly, for example, by conventional Southern blotting or Northern blotting to quantitate the transcription of mRNA (Thomas, 1980, Proc. Natl. Acad. Sci. USA, 77:5201-5205), dot blotting (DNA

analysis), or in situ hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies are employed that recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn are labeled and the assay
5 carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

In addition to the tissues discussed herein, biopsied tissue or peripheral blood can be conveniently assayed for the presence of cancer cells, including but not limited to prostate, testis, kidney, brain, bone, skin, ovarian, breast, pancreas, colon, lymphocytic
10 and lung cancers using for example, Northern, dot blot or RT-PCR analysis to detect 84P2A9 expression (see, e.g., FIGS 4-8). The presence of RT-PCR amplifiable 84P2A9 mRNA provides an indication of the presence of the cancer. RT-PCR detection assays for tumor cells in peripheral blood are currently being evaluated for use in the diagnosis and management of a number of human solid tumors. In the prostate cancer field, these
15 include RT-PCR assays for the detection of cells expressing PSA and PSM (Verkaik et al., 1997, Urol. Res. 25:373-384; Ghossein et al., 1995, J. Clin. Oncol. 13:1195-2000; Heston et al., 1995, Clin. Chem. 41:1687-1688). RT-PCR assays are well known in the art.

A related aspect of the invention is directed to predicting susceptibility to developing cancer in an individual. In one embodiment, a method for predicting
20 susceptibility to cancer comprises detecting 84P2A9 mRNA or 84P2A9 protein in a tissue sample, its presence indicating susceptibility to cancer, wherein the degree of 84P2A9 mRNA expression present correlates to the degree of susceptibility. In a specific embodiment, the presence of 84P2A9 in prostate or other tissue is examined, with the presence of 84P2A9 in the sample providing an indication of prostate cancer susceptibility
25 (or the emergence or existence of a prostate tumor). In a closely related embodiment, one can evaluate the integrity 84P2A9 nucleotide and amino acid sequences in a biological sample in order to identify perturbations in the structure of these molecules such as insertions, deletions, substitutions and the like, with the presence of one or more perturbations in 84P2A9 gene products in the sample providing an indication of cancer
30 susceptibility (or the emergence or existence of a tumor).

Another related aspect of the invention is directed to methods for gauging tumor aggressiveness. In one embodiment, a method for gauging aggressiveness of a tumor comprises determining the level of 84P2A9 mRNA or 84P2A9 protein expressed by cells in a sample of the tumor, comparing the level so determined to the level of 84P2A9 mRNA or 84P2A9 protein expressed in a corresponding normal tissue taken from the same individual or a normal tissue reference sample, wherein the degree of 84P2A9 mRNA or 84P2A9 protein expression in the tumor sample relative to the normal sample indicates the degree of aggressiveness. In a specific embodiment, aggressiveness of a tumor is evaluated by determining the extent to which 84P2A9 is expressed in the tumor cells, with higher expression levels indicating more aggressive tumors. In a closely related embodiment, one can evaluate the integrity of 84P2A9 nucleotide and amino acid sequences in a biological sample in order to identify perturbations in the structure of these molecules such as insertions, deletions, substitutions and the like, with the presence of one or more perturbations indicating more aggressive tumors.

Yet another related aspect of the invention is directed to methods for observing the progression of a malignancy in an individual over time. In one embodiment, methods for observing the progression of a malignancy in an individual over time comprise determining the level of 84P2A9 mRNA or 84P2A9 protein expressed by cells in a sample of the tumor, comparing the level so determined to the level of 84P2A9 mRNA or 84P2A9 protein expressed in an equivalent tissue sample taken from the same individual at a different time, wherein the degree of 84P2A9 mRNA or 84P2A9 protein expression in the tumor sample over time provides information on the progression of the cancer. In a specific embodiment, the progression of a cancer is evaluated by determining the extent to which 84P2A9 expression in the tumor cells alters over time, with higher expression levels indicating a progression of the cancer. Also, one can evaluate the integrity 84P2A9 nucleotide and amino acid sequences in a biological sample in order to identify perturbations in the structure of these molecules such as insertions, deletions, substitutions and the like, with the presence of one or more perturbations indicating a progression of the cancer.

The above diagnostic approaches can be combined with any one of a wide variety of prognostic and diagnostic protocols known in the art. For example, another

- embodiment of the invention disclosed herein is directed to methods for observing a coincidence between the expression of 84P2A9 gene and 84P2A9 gene products (or perturbations in 84P2A9 gene and 84P2A9 gene products) and a factor that is associated with malignancy as a means of diagnosing and prognosticating the status of a tissue sample.
- 5 In this context, a wide variety of factors associated with malignancy can be utilized such as the expression of genes associated with malignancy (e.g. PSA, PSCA and PSM expression for prostate cancer etc.) as well as gross cytological observations (see, e.g., Bocking et al., 1984, Anal. Quant. Cytol. 6(2):74-88; Eptstein, 1995, Hum. Pathol. 26(2):223-9; Thorson et al., 1998, Mod. Pathol. 11(6):543-51; Baisden et al., 1999, Am. J. Surg. Pathol. 10(8):918-24). Methods for observing a coincidence between the expression of 84P2A9 gene and 84P2A9 gene products (or perturbations in 84P2A9 gene and 84P2A9 gene products) and an additional factor that is associated with malignancy are useful, for example, because the presence of a set of specific factors that coincide with disease provides information crucial for diagnosing and prognosticating the status of a tissue
- 10 sample.

In a typical embodiment, methods for observing a coincidence between the expression of 84P2A9 gene and 84P2A9 gene products (or perturbations in 84P2A9 gene and 84P2A9 gene products) and a factor that is associated with malignancy entails detecting the overexpression of 84P2A9 mRNA or protein in a tissue sample, detecting the overexpression of PSA mRNA or protein in a tissue sample, and observing a coincidence of 84P2A9 mRNA or protein and PSA mRNA or protein overexpression. In a specific embodiment, the expression of 84P2A9 and PSA mRNA in prostate tissue is examined. In a preferred embodiment, the coincidence of 84P2A9 and PSA mRNA overexpression in the sample provides an indication of prostate cancer, prostate cancer susceptibility or the emergence or status of a prostate tumor.

Methods for detecting and quantifying the expression of 84P2A9 mRNA or protein are described herein and use of standard nucleic acid and protein detection and quantification technologies is well known in the art. Standard methods for the detection and quantification of 84P2A9 mRNA include in situ hybridization using labeled 84P2A9 riboprobes, Northern blot and related techniques using 84P2A9 polynucleotide probes, RT-PCR analysis using primers specific for 84P2A9, and other amplification type detection

methods, such as, for example, branched DNA, SISBA, TMA and the like. In a specific embodiment, semi-quantitative RT-PCR is used to detect and quantify 84P2A9 mRNA expression as described in the Examples that follow. Any number of primers capable of amplifying 84P2A9 can be used for this purpose, including but not limited to the various 5 primer sets specifically described herein. Standard methods for the detection and quantification of protein are used for this purpose. In a specific embodiment, polyclonal or monoclonal antibodies specifically reactive with the wild-type 84P2A9 protein can be used in an immunohistochemical assay of biopsied tissue.

10 **IDENTIFYING MOLECULES THAT INTERACT WITH 84P2A9**

The 84P2A9 protein sequences disclosed herein allow the skilled artisan to identify proteins, small molecules and other agents that interact with 84P2A9 and pathways activated by 84P2A9 via any one of a variety of art accepted protocols. For example, one can utilize one of the variety of so-called interaction trap systems (also 15 referred to as the "two-hybrid assay"). In such systems, molecules that interact reconstitute a transcription factor which directs expression of a reporter gene, whereupon the expression of the reporter gene is assayed. Typical systems identify protein-protein interactions in vivo through reconstitution of a eukaryotic transcriptional activator and are disclosed for example in U.S. Patent Nos. 5,955,280, 5,925,523, 20 5,846,722 and 6,004,746.

Alternatively one can identify molecules that interact with 84P2A9 protein sequences by screening peptide libraries. In such methods, peptides that bind to selected receptor molecules such as 84P2A9 are identified by screening libraries that encode a random or controlled collection of amino acids. Peptides encoded by the libraries are 25 expressed as fusion proteins of bacteriophage coat proteins, the bacteriophage particles are then screened against the receptors of interest.

Accordingly, peptides having a wide variety of uses, such as therapeutic or diagnostic reagents, can thus be identified without any prior information on the structure of the expected ligand or receptor molecule. Typical peptide libraries and screening

methods that can be used to identify molecules that interact with 84P2A9 protein sequences are disclosed for example in U.S. Patent Nos. 5,723,286 and 5,733,731.

Alternatively, cell lines that express 84P2A9 are used to identify protein-protein interactions mediated by 84P2A9. Such interactions can be examined using 5 immunoprecipitation techniques as shown by others (Hamilton BJ, et al. Biochem. Biophys. Res. Commun. 1999, 261:646-51). Typically 84P2A9 protein can be immunoprecipitated from 84P2A9 expressing prostate cancer cell lines using anti-84P2A9 antibodies. Alternatively, antibodies against His-tag can be used in a cell line engineered to express 84P2A9 (vectors mentioned above). The immunoprecipitated 10 complex can be examined for protein association by procedures such as Western blotting, ³⁵S-methionine labeling of proteins, protein microsequencing, silver staining and two dimensional gel electrophoresis.

Small molecules that interact with 84P2A9 can be identified through related 15 embodiments of such screening assays. For example, small molecules can be identified that interfere with protein function, including molecules that interfere with 84P2A9's ability to mediate phosphorylation and de-phosphorylation, second messenger signaling and tumorigenesis. Typical methods are discussed for example in U.S. Patent No. 5,928,868 and include methods for forming hybrid ligands in which at least one ligand is a small molecule. In an illustrative embodiment, the hybrid ligand is introduced into cells 20 that in turn contain a first and a second expression vector. Each expression vector includes DNA for expressing a hybrid protein that encodes a target protein linked to a coding sequence for a transcriptional module. The cells further contain a reporter gene, the expression of which is conditioned on the proximity of the first and second hybrid proteins to each other, an event that occurs only if the hybrid ligand binds to target sites 25 on both hybrid proteins. Those cells that express the reporter gene are selected and the unknown small molecule or the unknown hybrid protein is identified.

A typical embodiment of this invention consists of a method of screening for a molecule that interacts with an 84P2A9 amino acid sequence shown in FIG. 1 (SEQ ID NO: 2), comprising the steps of contacting a population of molecules with the 84P2A9 30 amino acid sequence, allowing the population of molecules and the 84P2A9 amino acid sequence to interact under conditions that facilitate an interaction, determining the

presence of a molecule that interacts with the 84P2A9 amino acid sequence and then separating molecules that do not interact with the 84P2A9 amino acid sequence from molecules that do interact with the 84P2A9 amino acid sequence. In a specific embodiment, the method further includes purifying a molecule that interacts with the
5 84P2A9 amino acid sequence. In a preferred embodiment, the 84P2A9 amino acid sequence is contacted with a library of peptides.

THERAPEUTIC METHODS AND COMPOSITIONS

The identification of 84P2A9 as a protein that is normally prostate and testis-related and which is also expressed in cancers of the prostate (and other cancers), opens
10 a number of therapeutic approaches to the treatment of such cancers. As discussed herein, it is possible that 84P2A9 functions as a transcription factor involved in activating tumor-promoting genes or repressing genes that block tumorigenesis.

The expression profile of 84P2A9 is reminiscent of the Cancer-Testis (CT) antigens or MAGE antigens, which are testis-related genes that are up-regulated in
15 melanomas and other cancers (Van den Eynde and Boon, Int J Clin Lab Res. 27:81-86, 1997). Due to their tissue-specific expression and high expression levels in cancer, the MAGE antigens are currently being investigated as targets for cancer vaccines (Durrant, Anticancer Drugs 8:727-733, 1997; Reynolds et al., Int J Cancer 72:972-976, 1997). The expression pattern of 84P2A9 provides evidence that it is likewise an ideal target for a
20 cancer vaccine approach to prostate cancer. Its structural features indicate that it may be a transcription factor, and provide evidence that 84P2A9 is a small molecule target.

Accordingly, therapeutic approaches aimed at inhibiting the activity of the 84P2A9 protein are expected to be useful for patients suffering from prostate cancer, testicular cancer, and other cancers expressing 84P2A9. These therapeutic approaches
25 generally fall into two classes. One class comprises various methods for inhibiting the binding or association of the 84P2A9 protein with its binding partner or with others proteins. Another class comprises a variety of methods for inhibiting the transcription of the 84P2A9 gene or translation of 84P2A9 mRNA.

84P2A9 as a Target for Antibody-Based Therapy

The structural features of 84P2A9 indicate that this molecule is an attractive target for antibody-based therapeutic strategies. A number of typical antibody strategies are known in the art for targeting both extracellular and intracellular molecules (see, e.g., complement and ADCC mediated killing as well as the use of intrabodies discussed herein). Because 84P2A9 is expressed by cancer cells of various lineages and not by corresponding normal cells, systemic administration of 84P2A9-immunoreactive compositions would be expected to exhibit excellent sensitivity without toxic, non-specific and/or non-target effects caused by binding of the immunotherapeutic molecule to non-target organs and tissues. Antibodies specifically reactive with domains of 84P2A9 can be useful to treat 84P2A9-expressing cancers systemically, either as conjugates with a toxin or therapeutic agent, or as naked antibodies capable of inhibiting cell proliferation or function.

84P2A9 antibodies can be introduced into a patient such that the antibody binds to 84P2A9 and modulates or perturbs a function such as an interaction with a binding partner and consequently mediates growth inhibition and/or destruction of the tumor cells and/or inhibits the growth of the tumor cells. Mechanisms by which such antibodies exert a therapeutic effect can include complement-mediated cytolysis, antibody-dependent cellular cytotoxicity, modulating the physiological function of 84P2A9, inhibiting ligand binding or signal transduction pathways, modulating tumor cell differentiation, altering tumor angiogenesis factor profiles, and/or by inducing apoptosis.

Those skilled in the art understand that antibodies can be used to specifically target and bind immunogenic molecules such as an immunogenic region of the 84P2A9 sequence shown in Figure 1. In addition, skilled artisans understand that it is routine to conjugate antibodies to cytotoxic agents. In this context, skilled artisans understand that when cytotoxic and/or therapeutic agents are delivered directly to cells by conjugating them to antibodies specific for a molecule expressed by that cell (e.g. 84P2A9), it is reasonable to expect that the cytotoxic agent will exert its known biological effect (e.g. cytotoxicity) on those cells.

A wide variety of compositions and methods for using antibodies conjugated to cytotoxic agents to kill cells are known in the art. In the context of cancers, typical methods entail administering to an animal having a tumor a biologically effective amount

of a conjugate comprising a selected cytotoxic and/or therapeutic agent linked to a targeting agent (e.g. an anti-84P2A9 antibody) that binds to a marker (e.g. 84P2A9) expressed, accessible to binding or localized on the cell surfaces. A typical embodiment consists of a method of delivering a cytotoxic and/or therapeutic agent to a cell
5 expressing 84P2A9 comprising conjugating the cytotoxic agent to an antibody that immunospecifically binds to an 84P2A9 epitope and exposing the cell to the antibody-agent conjugate. Another specific illustrative embodiment consists of a method of treating an individual suspected of suffering from metastasized cancer comprising the step of administering parenterally to said individual a pharmaceutical composition
10 comprising a therapeutically effective amount of an antibody conjugated to a cytotoxic and/or therapeutic agent.

Cancer immunotherapy using anti-84P2A9 antibodies may follow the teachings generated from various approaches that have been successfully employed in the treatment of other types of cancer, including but not limited to colon cancer (Arlen et al.,
15 1998, Crit. Rev. Immunol. 18:133-138), multiple myeloma (Ozaki et al., 1997, Blood 90:3179-3186; Tsunenari et al., 1997, Blood 90:2437-2444), gastric cancer (Kasprzyk et al., 1992, Cancer Res. 52:2771-2776), B-cell lymphoma (Funakoshi et al., 1996, J. Immunother. Emphasis Tumor Immunol. 19:93-101), leukemia (Zhong et al., 1996, Leuk. Res. 20:581-589), colorectal cancer (Moun et al., 1994, Cancer Res. 54:6160-6166;
20 Velders et al., 1995, Cancer Res. 55:4398-4403), and breast cancer (Shepard et al., 1991, J. Clin. Immunol. 11:117-127). Some therapeutic approaches involve conjugation of naked antibody to a toxin, such as the conjugation of ^{131}I to anti-CD20 antibodies (e.g., RituxanTM, IDEC Pharmaceuticals Corp.), while others involve co-administration of antibodies and other therapeutic agents, such as HerceptinTM (trastuzumab) with
25 paclitaxel (Genentech, Inc.). For treatment of prostate cancer, for example, 84P2A9 antibodies can be administered in conjunction with radiation, chemotherapy or hormone ablation.

Although 84P2A9 antibody therapy is useful for all stages of cancer, antibody therapy is particularly appropriate in advanced or metastatic cancers. Treatment with the
30 antibody therapy of the invention is indicated for patients who have received one or more rounds of chemotherapy, while combining the antibody therapy of the invention

with a chemotherapeutic or radiation regimen is preferred for patients who have not received chemotherapeutic treatment. Additionally, antibody therapy can enable the use of reduced dosages of concomitant chemotherapy, particularly for patients who do not tolerate the toxicity of the chemotherapeutic agent very well.

5 It is desirable for some cancer patients to be evaluated for the presence and level of 84P2A9 expression, preferably using immunohistochemical assessments of tumor tissue, quantitative 84P2A9 imaging, or other techniques capable of reliably indicating the presence and degree of 84P2A9 expression. Immunohistochemical analysis of tumor biopsies or surgical specimens is preferred for this purpose. Methods for
10 immunohistochemical analysis of tumor tissues are well known in the art.

Anti-84P2A9 monoclonal antibodies useful in treating prostate and other cancers include those that are capable of initiating a potent immune response against the tumor or those that are directly cytotoxic. In this regard, anti-84P2A9 monoclonal antibodies (mAbs) can elicit tumor cell lysis by either complement-mediated or antibody-dependent
15 cell cytotoxicity (ADCC) mechanisms, both of which require an intact Fc portion of the immunoglobulin molecule for interaction with effector cell Fc receptor sites or complement proteins. In addition, anti-84P2A9 mAbs that exert a direct biological effect on tumor growth are useful in the practice of the invention. Potential mechanisms by which such directly cytotoxic mAbs can act include inhibition of cell growth, modulation
20 of cellular differentiation, modulation of tumor angiogenesis factor profiles, and the induction of apoptosis. The mechanism by which a particular anti-84P2A9 mAb exerts an anti-tumor effect is evaluated using any number of in vitro assays designed to determine cell death such as ADCC, ADMMC, complement-mediated cell lysis, and so forth, as is generally known in the art.

25 The use of murine or other non-human monoclonal antibodies, or human/mouse chimeric mAbs can induce moderate to strong immune responses in some patients. In some cases, this will result in clearance of the antibody from circulation and reduced efficacy. In the most severe cases, such an immune response can lead to the extensive formation of immune complexes which, potentially, can cause renal
30 failure. Accordingly, preferred monoclonal antibodies used in the practice of the therapeutic methods of the invention are those that are either fully human or humanized

and that bind specifically to the target 84P2A9 antigen with high affinity but exhibit low or no antigenicity in the patient.

Therapeutic methods of the invention contemplate the administration of single anti-84P2A9 mAbs as well as combinations, or cocktails, of different mAbs. Such mAb
5 cocktails can have certain advantages inasmuch as they contain mAbs that target different epitopes, exploit different effector mechanisms or combine directly cytotoxic mAbs with mAbs that rely on immune effector functionality. Such mAbs in combination can exhibit synergistic therapeutic effects. In addition, the administration of anti-84P2A9 mAbs can be combined with other therapeutic agents, including but not
10 limited to various chemotherapeutic agents, androgen-blockers, and immune modulators (e.g., IL-2, GM-CSF). The anti-84P2A9 mAbs are administered in their "naked" or unconjugated form, or can have therapeutic agents conjugated to them.

The anti-84P2A9 antibody formulations are administered via any route capable of delivering the antibodies to the tumor site. Potentially effective routes of administration
15 include, but are not limited to, intravenous, intraperitoneal, intramuscular, intratumor, intradermal, and the like. Treatment will generally involve the repeated administration of the anti-84P2A9 antibody preparation via an acceptable route of administration such as intravenous injection (IV), typically at a dose in the range of about 0.1 to about 10 mg/kg body weight. Doses in the range of 10-500 mg mAb per week are effective and
20 well tolerated.

Based on clinical experience with the Herceptin mAb in the treatment of metastatic breast cancer, an initial loading dose of approximately 4 mg/kg patient body weight IV followed by weekly doses of about 2 mg/kg IV of the anti- 84P2A9 mAb preparation represents an acceptable dosing regimen. Preferably, the initial loading dose
25 is administered as a 90 minute or longer infusion. The periodic maintenance dose is administered as a 30 minute or longer infusion, provided the initial dose was well tolerated. However, as one of skill in the art will understand, various factors will influence the ideal dose regimen in a particular case. Such factors can include, for example, the binding affinity and half life of the Ab or mAbs used, the degree of 84P2A9
30 expression in the patient, the extent of circulating shed 84P2A9 antigen, the desired steady-state antibody concentration level, frequency of treatment, and the influence of

chemotherapeutic agents used in combination with the treatment method of the invention, as well as the health status of a particular patient.

5 Optionally, patients should be evaluated for the levels of 84P2A9 in a given sample (e.g. the levels of circulating 84P2A9 antigen and/or 84P2A9 expressing cells) in order to assist in the determination of the most effective dosing regimen and related factors. Such evaluations are also be used for monitoring purposes throughout therapy, and are useful to gauge therapeutic success in combination with evaluating other parameters (such as serum PSA levels in prostate cancer therapy).

10 **Inhibition of 84P2A9 Protein Function**

Within the first class of therapeutic approaches, the invention includes various methods and compositions for inhibiting the binding of 84P2A9 to its binding partner or its association with other protein(s) as well as methods for inhibiting 84P2A9 function.

15 ***Inhibition of 84P2A9 With Intracellular Antibodies***

In one approach, recombinant vectors encoding single chain antibodies that specifically bind to 84P2A9 are introduced into 84P2A9 expressing cells via gene transfer technologies, wherein the encoded single chain anti-84P2A9 antibody is expressed intracellularly, binds to 84P2A9 protein, and thereby inhibits its function. Methods for engineering such intracellular single chain antibodies are well known. Such intracellular antibodies, also known as "intrabodies", are specifically targeted to a particular compartment within the cell, providing control over where the inhibitory activity of the treatment will be focused. This technology has been successfully applied in the art (for review, see Richardson and Marasco, 1995, TIBTECH vol. 13). Intrabodies have been shown to virtually eliminate the expression of otherwise abundant cell surface receptors. See, for example, Richardson et al., 1995, Proc. Natl. Acad. Sci. USA 92: 3137-3141; Beerli et al., 1994, J. Biol. Chem. 269: 23931-23936; Deshane et al., 1994, Gene Ther. 1: 332-337.

30 Single chain antibodies comprise the variable domains of the heavy and light chain joined by a flexible linker polypeptide, and are expressed as a single polypeptide.

Optionally, single chain antibodies are expressed as a single chain variable region fragment joined to the light chain constant region. Well known intracellular trafficking signals are engineered into recombinant polynucleotide vectors encoding such single chain antibodies in order to precisely target the expressed intrabody to the desired 5 intracellular compartment. For example, intrabodies targeted to the endoplasmic reticulum (ER) are engineered to incorporate a leader peptide and, optionally, a C-terminal ER retention signal, such as the KDEL amino acid motif. Intrabodies intended to exert activity in the nucleus are engineered to include a nuclear localization signal. Lipid moieties are joined to intrabodies in order to tether the intrabody to the cytosolic 10 side of the plasma membrane. Intrabodies can also be targeted to exert function in the cytosol. For example, cytosolic intrabodies are used to sequester factors within the cytosol, thereby preventing them from being transported to their natural cellular destination.

In one embodiment, intrabodies are used to capture 84P2A9 in the nucleus, 15 thereby preventing its activity within the nucleus. Nuclear targeting signals are engineered into such 84P2A9 intrabodies in order to achieve the desired targeting. Such 84P2A9 intrabodies are designed to bind specifically to a particular 84P2A9 domain. In another embodiment, cytosolic intrabodies that specifically bind to the 84P2A9 protein are used to prevent 84P2A9 from gaining access to the nucleus, thereby preventing it 20 from exerting any biological activity within the nucleus (e.g., preventing 84P2A9 from forming transcription complexes with other factors).

In order to specifically direct the expression of such intrabodies to particular cells, the transcription of the intrabody is placed under the regulatory control of an appropriate tumor-specific promoter and/or enhancer. In order to target intrabody 25 expression specifically to prostate, for example, the PSA promoter and/or promoter/enhancer can be utilized (See, for example, U.S. Patent No. 5,919,652).

Inhibition of 84P2A9 With Recombinant Proteins

In another approach, recombinant molecules that bind to 84P2A9 thereby 30 prevent or inhibit 84P2A9 from accessing/binding to its binding partner(s) or associating

with other protein(s) are used to inhibit 84P2A9 function. Such recombinant molecules can, for example, contain the reactive part(s) of an 84P2A9 specific antibody molecule. In a particular embodiment, the 84P2A9 binding domain of an 84P2A9 binding partner is engineered into a dimeric fusion protein comprising two 84P2A9 ligand binding domains
5 linked to the Fc portion of a human IgG, such as human IgG1. Such IgG portion can contain, for example, the C_H2 and C_H3 domains and the hinge region, but not the C_H1 domain. Such dimeric fusion proteins are administered in soluble form to patients suffering from a cancer associated with the expression of 84P2A9, including but not limited to prostate and testicular cancers, where the dimeric fusion protein specifically binds to
10 84P2A9 thereby blocking 84P2A9 interaction with a binding partner. Such dimeric fusion proteins are further combined into multimeric proteins using known antibody linking technologies.

Inhibition of 84P2A9 Transcription or Translation

15 Within the second class of therapeutic approaches, the invention provides various methods and compositions for inhibiting the transcription of the 84P2A9 gene. Similarly, the invention also provides methods and compositions for inhibiting the translation of 84P2A9 mRNA into protein.

In one approach, a method of inhibiting the transcription of the 84P2A9 gene
20 comprises contacting the 84P2A9 gene with an 84P2A9 antisense polynucleotide. In another approach, a method of inhibiting 84P2A9 mRNA translation comprises contacting the 84P2A9 mRNA with an antisense polynucleotide. In another approach, an 84P2A9 specific ribozyme is used to cleave the 84P2A9 message, thereby inhibiting translation. Such antisense and ribozyme based methods can also be directed to the
25 regulatory regions of the 84P2A9 gene, such as the 84P2A9 promoter and/or enhancer elements. Similarly, proteins capable of inhibiting an 84P2A9 gene transcription factor can be used to inhibit 84P2A9 mRNA transcription. The various polynucleotides and compositions useful in the aforementioned methods have been described above. The use of antisense and ribozyme molecules to inhibit transcription and translation is well
30 known in the art.

Other factors that inhibit the transcription of 84P2A9 through interfering with 84P2A9 transcriptional activation are also useful for the treatment of cancers expressing 84P2A9. Similarly, factors that are capable of interfering with 84P2A9 processing are useful for the treatment of cancers expressing 84P2A9. Cancer treatment methods utilizing such factors are also within the scope of the invention.

General Considerations for Therapeutic Strategies

Gene transfer and gene therapy technologies can be used for delivering therapeutic polynucleotide molecules to tumor cells synthesizing 84P2A9 (i.e., antisense, ribozyme, 10 polynucleotides encoding intrabodies and other 84P2A9 inhibitory molecules). A number of gene therapy approaches are known in the art. Recombinant vectors encoding 84P2A9 antisense polynucleotides, ribozymes, factors capable of interfering with 84P2A9 transcription, and so forth, can be delivered to target tumor cells using such gene therapy approaches.

15 The above therapeutic approaches can be combined with any one of a wide variety of surgical, chemotherapy or radiation therapy regimens. These therapeutic approaches can enable the use of reduced dosages of chemotherapy and/or less frequent administration, an advantage for all patients and particularly for those that do not tolerate the toxicity of the chemotherapeutic agent well.

20 The anti-tumor activity of a particular composition (e.g., antisense, ribozyme, intrabody), or a combination of such compositions, can be evaluated using various in vitro and in vivo assay systems. In vitro assays for evaluating therapeutic potential include cell growth assays, soft agar assays and other assays indicative of tumor promoting activity, binding assays capable of determining the extent to which a therapeutic composition will 25 inhibit the binding of 84P2A9 to a binding partner, etc.

In vivo, the effect of an 84P2A9 therapeutic composition can be evaluated in a suitable animal model. For example, xenogenic prostate cancer models wherein human prostate cancer explants or passaged xenograft tissues are introduced into immune compromised animals, such as nude or SCID mice, are appropriate in relation to prostate 30 cancer and have been described (Klein et al., 1997, Nature Medicine 3: 402-408). For

example, PCT Patent Application WO98/16628, Sawyers et al., published April 23, 1998, describes various xenograft models of human prostate cancer capable of recapitulating the development of primary tumors, micrometastasis, and the formation of osteoblastic metastases characteristic of late stage disease. Efficacy can be predicted using assays that
5 measure inhibition of tumor formation, tumor regression or metastasis, and the like. See, also, the Examples below.

In vivo assays that evaluate the promotion of apoptosis are useful in evaluating therapeutic compositions. In one embodiment, xenografts from tumor bearing mice treated with the therapeutic composition can be examined for the presence of apoptotic
10 foci and compared to untreated control xenograft-bearing mice. The extent to which apoptotic foci are found in the tumors of the treated mice provides an indication of the therapeutic efficacy of the composition.

The therapeutic compositions used in the practice of the foregoing methods can be formulated into pharmaceutical compositions comprising a carrier suitable for the desired
15 delivery method. Suitable carriers include any material that when combined with the therapeutic composition retains the anti-tumor function of the therapeutic composition and is generally non-reactive with the patient's immune system. Examples include, but are not limited to, any of a number of standard pharmaceutical carriers such as sterile phosphate buffered saline solutions, bacteriostatic water, and the like (see, generally,
20 Remington's Pharmaceutical Sciences 16th Edition, A. Osal, Ed., 1980).

Therapeutic formulations can be solubilized and administered via any route capable of delivering the therapeutic composition to the tumor site. Potentially effective routes of administration include, but are not limited to, intravenous, parenteral, intraperitoneal, intramuscular, intratumor, intradermal, intraorgan, orthotopic, and the
25 like. A preferred formulation for intravenous injection comprises the therapeutic composition in a solution of preserved bacteriostatic water, sterile unpreserved water, and/or diluted in polyvinylchloride or polyethylene bags containing 0.9% sterile Sodium Chloride for Injection, USP. Therapeutic protein preparations can be lyophilized and stored as sterile powders, preferably under vacuum, and then reconstituted in
30 bacteriostatic water containing, for example, benzyl alcohol preservative, or in sterile water prior to injection.

Dosages and administration protocols for the treatment of cancers using the foregoing methods will vary with the method and the target cancer, and will generally depend on a number of other factors appreciated in the art.

5 CANCER VACCINES

As noted above, the expression profile of 84P2A9 shows that it is highly expressed in advanced and metastasized prostate cancer. This expression pattern is reminiscent of the Cancer-Testis (CT) antigens or MAGE antigens, which are testis-specific genes that are up-regulated in melanomas and other cancers (Van den Eynde and
10 Boon, Int J Clin Lab Res. 27:81-86, 1997). Due to their tissue-specific expression and high expression levels in cancer, the MAGE antigens are currently being investigated as targets for cancer vaccines (Durrant, Anticancer Drugs 8:727-733, 1997; Reynolds et al., Int J Cancer 72:972-976, 1997).

The invention further provides cancer vaccines comprising an 84P2A9-related
15 protein or fragment as well as DNA based vaccines. In view of the expression of 84P2A9, cancer vaccines are effective at specifically preventing and/or treating 84P2A9 expressing cancers without creating non-specific effects on non-target tissues. The use of a tumor antigen in a vaccine for generating humoral and cell-mediated immunity for use in anti-cancer therapy is well known in the art and has been employed in prostate cancer using
20 human PSMA and rodent PAP immunogens (Hodge et al., 1995, Int. J. Cancer 63:231-237; Fong et al., 1997, J. Immunol. 159:3113-3117).

Such methods can be readily practiced by employing an 84P2A9 protein, or fragment thereof, or an 84P2A9-encoding nucleic acid molecule and recombinant vectors capable of expressing and appropriately presenting the 84P2A9 immunogen
25 (which typically comprises a number of humoral or T cell epitopes immunoreactive epitopes). In this context, skilled artisans understand that a wide variety of different vaccine systems for delivery of immunoreactive epitopes are known in the art (see, e.g., Heryln et al., Ann Med 1999 Feb;31(1):66-78; Maruyama et al., Cancer Immunol Immunother 2000 Jun;49(3):123-32) Briefly, such techniques consists of methods of
30 generating an immune response (e.g. a humoral and/or cell mediated response) in a

mammal comprising the steps exposing the mammal's immune system to an immunoreactive epitope (e.g. an epitope of the 84P2A9 protein shown in SEQ ID NO: 2) so that the mammal generates an immune response that is specific for that epitope (e.g. generates antibodies that specifically recognize that epitope). In a preferred method, 5 the 84P2A9 immunogen contains a biological motif. In a highly preferred embodiment, the 84P2A9 immunogen contains one or more amino acid sequences identified using one of the pertinent analytical techniques well known in the art such as the sequences shown in Table 1.

A wide variety of methods for generating an immune response in a mammal are 10 well known in the art (for example as the first step in the generation of hybridomas). Methods of generating an immune response in a mammal comprise exposing the mammal's immune system to an exogenous immunogenic epitope on a protein (e.g. the 84P2A9 protein of SEQ ID NO: 2) so that an immune response is generated. A typical embodiment consists of a method for generating an immune response to 84P2A9 in a 15 host, by contacting the host with a sufficient amount of 84P2A9 or a B cell or cytotoxic T-cell eliciting epitope or analog thereof, and at least one periodic interval thereafter contacting the host with additional 84P2A9 or a B cell or cytotoxic T-cell eliciting epitope or analog thereof. A specific embodiment consists of a method of generating an 20 immune response against an 84P2A9 protein or a multiepitopic peptide comprising administering 84P2A9 immunogen (e.g. the 84P2A9 protein or a peptide fragment 25 thereof, an 84P2A9 fusion protein etc.) in a vaccine preparation to humans or animals. Typically, such vaccine preparations further contain a suitable adjuvant. (see, e.g., U.S. Patent No. 6,146,635). A representative variation on these methods consists of a method 30 of generating an immune response in an individual against an 84P2A9 immunogen comprising administering in vivo to muscle or skin of the individual's body a genetic vaccine facilitator such as one selected from the group consisting of: anionic lipids; saponins; lectins; estrogenic compounds; hydroxylated lower alkyls; dimethyl sulfoxide; and urea; and a DNA molecule that is dissociated from an infectious agent and comprises a DNA sequence that encodes the 84P2A9 immunogen, the DNA sequence operatively linked to regulatory sequences which control the expression of the DNA 35 sequence; wherein the DNA molecule is taken up by cells, the DNA sequence is

expressed in the cells and an immune response is generated against the immunogen. (see, e.g., U.S. Patent No. 5,962,428).

- In an illustrative example of a specific method for generating an immune response, viral gene delivery systems are used to deliver an 84P2A9-encoding nucleic acid molecule.
- 5 Various viral gene delivery systems that can be used in the practice of this aspect of the invention include, but are not limited to, vaccinia, fowlpox, canarypox, adenovirus, influenza, poliovirus, adeno-associated virus, lentivirus, and sindbus virus (Restifo, 1996, Curr. Opin. Immunol. 8:658-663). Non-viral delivery systems can also be employed by using naked DNA encoding an 84P2A9 protein or fragment thereof introduced into the
- 10 patient (e.g., intramuscularly or intradermally) to induce an anti-tumor response. In one embodiment, the full-length human 84P2A9 cDNA is employed. In another embodiment, 84P2A9 nucleic acid molecules encoding specific cytotoxic T lymphocyte (CTL) epitopes can be employed. CTL epitopes can be determined using specific algorithms (e.g., Epimer, Brown University) to identify peptides within an 84P2A9 protein that are capable of
- 15 optimally binding to specified HLA alleles.

- Various ex vivo strategies can also be employed. One approach involves the use of dendritic cells to present 84P2A9 antigen to a patient's immune system. Dendritic cells express MHC class I and II molecules, B7 co-stimulator, and IL-12, and are thus highly specialized antigen presenting cells. In prostate cancer, autologous dendritic cells pulsed
- 20 with peptides of the prostate-specific membrane antigen (PSMA) are being used in a Phase I clinical trial to stimulate prostate cancer patients' immune systems (Tjoa et al., 1996, Prostate 28:65-69; Murphy et al., 1996, Prostate 29:371-380). Thus, dendritic cells can be used to present 84P2A9 peptides to T cells in the context of MHC class I and II molecules. In one embodiment, autologous dendritic cells are pulsed with 84P2A9
- 25 peptides capable of binding to MHC class I and/or class II molecules. In another embodiment, dendritic cells are pulsed with the complete 84P2A9 protein. Yet another embodiment involves engineering the overexpression of the 84P2A9 gene in dendritic cells using various implementing vectors known in the art, such as adenovirus (Arthur et al., 1997, Cancer Gene Ther. 4:17-25), retrovirus (Henderson et al., 1996, Cancer Res. 30:3763-3770), lentivirus, adeno-associated virus, DNA transfection (Ribas et al., 1997, Cancer Res. 57:2865-2869), or tumor-derived RNA transfection (Ashley et al., 1997, J.

Exp. Med. 186:1177-1182). Cells expressing 84P2A9 can also be engineered to express immune modulators, such as GM-CSF, and used as immunizing agents.

- Anti-idiotypic anti-84P2A9 antibodies can also be used in anti-cancer therapy as a vaccine for inducing an immune response to cells expressing an 84P2A9 protein.
- 5 Specifically, the generation of anti-idiotypic antibodies is well known in the art and can readily be adapted to generate anti-idiotypic anti-84P2A9 antibodies that mimic an epitope on an 84P2A9 protein (see, for example, Wagner et al., 1997, Hybridoma 16: 33-40; Foon et al., 1995, J. Clin. Invest. 96:334-342; Herlyn et al., 1996, Cancer Immunol. Immunother. 43:65-76). Such an anti-idiotypic antibody can be used in cancer vaccine strategies.
- 10 Genetic immunization methods can be employed to generate prophylactic or therapeutic humoral and cellular immune responses directed against cancer cells expressing 84P2A9. Constructs comprising DNA encoding an 84P2A9-related protein/immunogen and appropriate regulatory sequences can be injected directly into muscle or skin of an individual, such that the cells of the muscle or skin take-up the construct and express the
- 15 encoded 84P2A9 protein/immunogen. Alternatively, a vaccine comprises an 84P2A9-related protein. Expression of the 84P2A9 protein immunogen results in the generation of prophylactic or therapeutic humoral and cellular immunity against bone, colon, pancreatic, testicular, cervical and ovarian cancers. Various prophylactic and therapeutic genetic immunization techniques known in the art can be used (for review, see information and
- 20 references published at Internet address www.genweb.com).

KITS

- For use in the diagnostic and therapeutic applications described herein, kits are also provided by the invention. Such kits can comprise a carrier being compartmentalized to receive in close confinement one or more containers such as vials, tubes, and the like,
- 25 each of the container(s) comprising one of the separate elements to be used in the method. For example, the container(s) can comprise a probe that is or can be detectably labeled. Such probe can be an antibody or polynucleotide specific for an 84P2A9-related protein or an 84P2A9 gene or message, respectively. Where the kit utilizes nucleic acid hybridization to detect the target nucleic acid, the kit can also have containers containing
- 30 nucleotide(s) for amplification of the target nucleic acid sequence and/or a container

comprising a reporter-means, such as a biotin-binding protein, such as avidin or streptavidin, bound to a reporter molecule, such as an enzymatic, florescent, or radioisotope label.

5 The kit of the invention will typically comprise the container described above and one or more other containers comprising materials desirable from a commercial and user standpoint, including buffers, diluents, filters, needles, syringes, and package inserts with instructions for use. A label can be present on the container to indicate that the composition is used for a specific therapy or non-therapeutic application, and can also indicate directions for either in vivo or in vitro use, such as those described above.

10

p84P2A9-1 has been deposited under the requirements of the Budapest Treaty on January 6, 2000 with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA 20110-2209 USA, and have been identified as ATCC Accession No. PTA-1151.

15

EXAMPLES

Various aspects of the invention are further described and illustrated by way of the several examples that follow, none of which are intended to limit the scope of the
20 invention.

Example 1: SSH-Generated Isolation of cDNA Fragment of the 84P2A9 Gene

Materials and Methods

LAPC Xenografts and Human Tissues:

25 LAPC xenografts were obtained from Dr. Charles Sawyers (UCLA) and generated as described (Klein et al, 1997, Nature Med. 3: 402-408). Androgen dependent and independent LAPC-4 xenografts (LAPC-4 AD and AI, respectively) and LAPC-9 AD and AI xenografts were grown in male SCID mice and were passaged as small tissue chunks in recipient males. LAPC-4 and -9 AI xenografts were derived from LAPC-4 or

-9 AD tumors, respectively. Male mice bearing AD tumors were castrated and maintained for 2-3 months. After the tumors re-grew, the tumors were harvested and passaged in castrated males or in female SCID mice. Human tissues for RNA and protein analyses were obtained from the Human Tissue Resource Center (HTRC) at the 5 UCLA (Los Angeles, CA) and from QualTek, Inc. (Santa Barbara, CA). A benign prostatic hyperplasia tissue sample was patient-derived.

Cell Lines:

Human cell lines (e.g., HeLa) were obtained from the ATCC and were
10 maintained in DMEM with 5% fetal calf serum.

RNA Isolation:

Tumor tissue and cell lines were homogenized in Trizol reagent (Life Technologies, Gibco BRL) using 10 ml/ g tissue or 10 ml/ 10⁸ cells to isolate total RNA.
15 Poly A RNA was purified from total RNA using Qiagen's Oligotex mRNA Mini and Midi kits. Total and mRNA were quantified by spectrophotometric analysis (O.D. 260/280 nm) and analyzed by gel electrophoresis.

Oligonucleotides:

20 The following HPLC purified oligonucleotides were used.

DPNCDN (cDNA synthesis primer):

5'TTTT**GATCAAGCTT**₃₀3' (SEQ ID NO: 7)

25 Adaptor 1:

5'CTAATACGACTCACTATAGGGCTCGAGCGGCCGCCGGCAG3' (SEQ ID NO:
8)

3'GGCCCGTCCTAG5' (SEQ ID NO: 9)

Adaptor 2:

30 5'GTAATACGACTCACTATAGGGCAGCGTGGTGGCGCCGAG3' (SEQ ID NO:10)

3'CGGCTCCTAG5' (SEQ ID NO: 11)

PCR primer 1:

5'CTAATACGACTCACTATAAGGC3' (SEQ ID NO: 12)

5 Nested primer (NP)1:

5'TCGAGCGGCCGCCGGCAGGA3' (SEQ ID NO: 13)

Nested primer (NP)2:

5'AGCGTGGTCGCGGCCGAGGA3' (SEQ ID NO: 14)

10 Suppression Subtractive Hybridization:

Suppression Subtractive Hybridization (SSH) was used to identify cDNAs corresponding to genes that may be differentially expressed in prostate cancer. The SSH reaction utilized cDNA from two LAPC-4 AD xenografts. Specifically, the 84P2A9 SSH sequence was identified from a subtraction where cDNA derived from an LAPC-4 AD tumor, 3 days post-castration, was subtracted from cDNA derived from an LAPC-4 AD tumor grown in an intact male. The LAPC-4 AD xenograft tumor grown in an intact male was used as the source of the "tester" cDNA, while the cDNA from the LAPC-4 AD tumor, 3 days post-castration, was used as the source of the "driver" cDNA.

Double stranded cDNAs corresponding to tester and driver cDNAs were synthesized from 2 µg of poly(A)⁺ RNA isolated from the relevant xenograft tissue, as described above, using CLONTECH's PCR-Select cDNA Subtraction Kit and 1 ng of oligonucleotide DPNCDN as primer. First- and second-strand synthesis were carried out as described in the Kit's user manual protocol (CLONTECH Protocol No. PT1117-1, Catalog No. K1804-1). The resulting cDNA was digested with Dpn II for 3 hrs. at 25 37°C. Digested cDNA was extracted with phenol/chloroform (1:1) and ethanol precipitated.

Driver cDNA was generated by combining in a 1:1 ratio Dpn II digested cDNA from the relevant xenograft source (see above) with a mix of digested cDNAs derived from human benign prostatic hyperplasia (BPH), the human cell lines HeLa, 293, A431, 30 Colo205, and mouse liver.

Tester cDNA was generated by diluting 1 μ l of Dpn II digested cDNA from the relevant xenograft source (see above) (400 ng) in 5 μ l of water. The diluted cDNA (2 μ l, 160 ng) was then ligated to 2 μ l of Adaptor 1 and Adaptor 2 (10 μ M), in separate ligation reactions, in a total volume of 10 μ l at 16°C overnight, using 400 u of T4 DNA ligase 5 (CLONTECH). Ligation was terminated with 1 μ l of 0.2 M EDTA and heating at 72°C for 5 min.

The first hybridization was performed by adding 1.5 μ l (600 ng) of driver cDNA to each of two tubes containing 1.5 μ l (20 ng) Adaptor 1- and Adaptor 2- ligated tester cDNA. In a final volume of 4 μ l, the samples were overlaid with mineral oil, denatured 10 in an MJ Research thermal cycler at 98°C for 1.5 minutes, and then were allowed to hybridize for 8 hrs at 68°C. The two hybridizations were then mixed together with an additional 1 μ l of fresh denatured driver cDNA and were allowed to hybridize overnight at 68°C. The second hybridization was then diluted in 200 μ l of 20 mM Hepes, pH 8.3, 50 mM NaCl, 0.2 mM EDTA, heated at 70°C for 7 min. and stored at -20°C.

15

PCR Amplification, Cloning and Sequencing of Gene Fragments Generated from SSH:

To amplify gene fragments resulting from SSH reactions, two PCR amplifications were performed. In the primary PCR reaction 1 μ l of the diluted final hybridization mix was added to 1 μ l of PCR primer 1 (10 μ M), 0.5 μ l dNTP mix (10 μ M), 2.5 μ l 10 x 20 reaction buffer (CLONTECH) and 0.5 μ l 50 x Advantage cDNA polymerase Mix (CLONTECH) in a final volume of 25 μ l. PCR 1 was conducted using the following conditions: 75°C for 5 min., 94°C for 25 sec., then 27 cycles of 94°C for 10 sec, 66°C for 30 sec, 72°C for 1.5 min. Five separate primary PCR reactions were performed for each experiment. The products were pooled and diluted 1:10 with water. For the secondary 25 PCR reaction, 1 μ l from the pooled and diluted primary PCR reaction was added to the same reaction mix as used for PCR 1, except that primers NP1 and NP2 (10 μ M) were used instead of PCR primer 1. PCR 2 was performed using 10-12 cycles of 94°C for 10 sec, 68°C for 30 sec, 72°C for 1.5 minutes. The PCR products were analyzed using 2% agarose gel electrophoresis.

The PCR products were inserted into pCR2.1 using the T/A vector cloning kit (Invitrogen). Transformed E. coli were subjected to blue/white and ampicillin selection. White colonies were picked and arrayed into 96 well plates and were grown in liquid culture overnight. To identify inserts, PCR amplification was performed on 1 ml of bacterial culture using the conditions of PCR1 and NP1 and NP2 as primers. PCR products were analyzed using 2% agarose gel electrophoresis.

Bacterial clones were stored in 20% glycerol in a 96 well format. Plasmid DNA was prepared, sequenced, and subjected to nucleic acid homology searches of the GenBank, dBest, and NCI-CGAP databases.

10

RT-PCR Expression Analysis:

First strand cDNAs can be generated from 1 μ g of mRNA with oligo (dT)12-18 priming using the Gibco-BRL Superscript Preamplification system. The manufacturer's protocol can be used and included an incubation for 50 min at 42°C with reverse transcriptase followed by RNase H treatment at 37°C for 20 min. After completing the reaction, the volume can be increased to 200 μ l with water prior to normalization. First strand cDNAs from 16 different normal human tissues can be obtained from Clontech.

Normalization of the first strand cDNAs from multiple tissues can be performed by using the primers 5'atategcgcgegtcgctgcacaa3' (SEQ ID NO: 15) and 20 5'agcccacacgcgcgttcattttaggg 3' (SEQ ID NO: 16) to amplify β -actin. First strand cDNA (5 μ l) can be amplified in a total volume of 50 μ l containing 0.4 μ M primers, 0.2 μ M each dNTPs, 1XPCR buffer (Clontech, 10 mM Tris-HCL, 1.5 mM MgCl₂, 50 mM KCl, pH8.3) and 1X Klentaq DNA polymerase (Clontech). Five μ l of the PCR reaction can be removed at 18, 20, and 22 cycles and used for agarose gel electrophoresis. PCR 25 can be performed using an MJ Research thermal cycler under the following conditions: Initial denaturation can be at 94°C for 15 sec, followed by a 18, 20, and 22 cycles of 94°C for 15, 65°C for 2 min, 72°C for 5 sec. A final extension at 72°C can be carried out for 2 min. After agarose gel electrophoresis, the band intensities of the 283 bp β -actin bands from multiple tissues can be compared by visual inspection. Dilution factors for the first 30 strand cDNAs can be calculated to result in equal β -actin band intensities in all tissues

after 22 cycles of PCR. Three rounds of normalization can be required to achieve equal band intensities in all tissues after 22 cycles of PCR.

To determine expression levels of the 84P2A9 gene, 5 µl of normalized first strand cDNA can be analyzed by PCR using 25, 30, and 35 cycles of amplification using 5 primer pairs that can be designed with the assistance of (MIT; for details, see, www.genome.wi.mit.edu).

Semi quantitative expression analysis can be achieved by comparing the PCR products at cycle numbers that give light band intensities.

10 *Results*

Two SSH experiments described in the Materials and Methods, *supra*, led to the isolation of numerous candidate gene fragment clones (SSH clones). All candidate clones were sequenced and subjected to homology analysis against all sequences in the major public gene and EST databases in order to provide information on the identity of 15 the corresponding gene and to help guide the decision to analyze a particular gene for differential expression. In general, gene fragments that had no homology to any known sequence in any of the searched databases, and thus considered to represent novel genes, as well as gene fragments showing homology to previously sequenced expressed sequence tags (ESTs), were subjected to differential expression analysis by RT-PCR 20 and/or Northern analysis.

One of the SHH clones comprising about 425 bp, showed significant homology to several testis-derived ESTs but no homology to any known gene, and was designated 84P2A9.

Northern expression analysis of first strand cDNAs from 16 normal tissues 25 showed a highly prostate and testis-related expression pattern in adult tissues (FIG. 4).

Example 2: Full Length Cloning of 84P2A9

A full length 84P2A9 cDNA clone (clone 1) of 2347 base pairs (bp) was cloned from an LAPC-4 AD cDNA library (Lambda ZAP Express, Stratagene) (Fig. 2). The 30 cDNA encodes an open reading frame (ORF) of 504 amino acids. Sequence analysis

revealed the presence of six potential nuclear localization signals and is predicted to be nuclear using the PSORT program (<http://psort.nibb.ac.jp:8800/form.html>). The protein sequence is homologous to a human brain protein KIAA1152 (39.5% identity over a 337 amino acid region), and exhibits a domain that is homologous to the LUCA15
5 tumor suppressor protein (64.3% identity over a 42 amino acid region)(GenBank Accession #P52756)(Fig. 3). The 84P2A9 cDNA was deposited on January 5, 2000 with the American Type Culture Collection (ATCC; Manassas, VA) as plasmid p84P2A9-1, and has been assigned Accession No. PTA-1151.

The 84P2A9 proteins have no homology to any known proteins, but the
10 sequence does overlap with several ESTs derived from testis.

Example 3: 84P2A9 Gene Expression Analysis

84P2A9 mRNA expression in normal human tissues was analyzed by Northern blotting of two multiple tissue blots (Clontech; Palo Alto, California), comprising a total
15 of 16 different normal human tissues, using labeled 84P2A9 SSH fragment (Example 1) as a probe. RNA samples were quantitatively normalized with a β -actin probe. The results demonstrated expression of a 2.4 and 4.5 kb transcript in normal testis and prostate (Fig. 4).

To analyze 84P2A9 expression in prostate cancer tissues lines northern blotting
20 was performed on RNA derived from the LAPC xenografts. The results show high levels of 84P2A9 expression in all the xenografts, with the highest levels detected in LAPC-9 AD, LAPC-9 AI (Fig. 4 and Figure 5). These results provide evidence that 84P2A9 is up-regulated in prostate cancer.

In addition, high levels of expression were detected in brain (PFSK-1, T98G),
25 bone (HOS, U2-OS), lung (CALU-1, NCI-H82, NCI-H146), and kidney (769-P, A498, CAKI-1, SW839) cancer cell lines (Fig. 5). Moderate expression levels were detected in several pancreatic (PANC-1, BxPC-3, HPAC, CAPAN-1), colon (SK-CO-1, CACO-2, LOVO, COLO-205), bone (SK-ES-1, RD-ES), breast (MCF-7, MDA-MB-435s) and testicular cancer (NCCIT) cell lines (Fig. 5).

In addition, prostate cancer patient samples show expression of 84P2A9 in both the normal and the tumor part of the prostate tissues (Fig. 6). These results suggest that 84P2A9 is a very testis specific gene that is up-regulated in prostate cancer and potentially other cancers. Similar to the MAGE antigens, 84P2A9 may thus qualify as a

5 cancer-testis antigen (Van den Eynde and Boon, Int J Clin Lab Res. 27:81-86, 1997).

84P2A9 expression in normal tissues can be further analyzed using a multi-tissue RNA dot blot containing different samples (representing mainly normal tissues as well as a few cancer cell lines).

10 **Example 4: Generation of 84P2A9 Polyclonal Antibodies**

Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. Typically a peptide can be designed from a coding region of

15 84P2A9. Alternatively the immunizing agent may include all or portions of the 84P2A9 protein, or fusion proteins thereof. For example, the 84P2A9 amino acid sequence can be fused to any one of a variety of known fusion protein partners that are well known in the art such as maltose binding protein, LacZ, thioredoxin or an immunoglobulin constant region (see, e.g., Current Protocols In Molecular Biology, Volume 2, Unit 16,

20 Frederick M. Ausubel et al. eds., 1995; Linsley, P.S., Brady, W., Urnes, M., Grosmaire, L., Damle, N., and Ledbetter, L.(1991) J.Exp. Med. 174, 561-566). Other such recombinant bacterial proteins include glutathione-S-transferase (GST), and HIS tagged fusion proteins of 84P2A9 (which can be purified from induced bacteria using the appropriate affinity matrix).

25 It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. Examples of adjuvants which may be employed include Freund's complete adjuvant and MPL-TDM adjuvant

30 (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate).

In a typical protocol, rabbits can be initially immunized subcutaneously with about 200 µg of fusion protein or KLH-peptide mixed in complete Freund's adjuvant. Rabbits are then injected subcutaneously every two weeks with about 200 µg of immunogen in incomplete Freund's adjuvant. Test bleeds are taken approximately 7-10 days following each immunization and used to monitor the titer of the antiserum by ELISA.

Specificity of the antiserum is tested by Western blot and immunoprecipitation analyses using lysates of genetically engineered cells or cells expressing endogenous 84P2A9. To genetically engineer cells to express 84P2A9, the full length 84P2A9 cDNA can be cloned into an expression vector that provides a 6His tag at the carboxyl-terminus (pCDNA 3.1 myc-his, Invitrogen). After transfection of the constructs into 293T cells, cell lysates can be immunoprecipitated and Western blotted using anti-His or v5 anti-epitope antibody (Invitrogen) and the anti-84P2A9 serum (see, e.g., FIG. 11). Sera from His-tagged protein and peptide immunized rabbits as well as depleted GST and MBP protein sera are purified by passage over an affinity column composed of the respective immunogen covalently coupled to Affigel matrix (BioRad).

Example 5: Production of Recombinant 84P2A9 in Bacterial and Mammalian Systems

20 BACTERIAL CONSTRUCTS

Production of Recombinant 84P2A9 using pGEX Constructs

To express 84P2A9 in bacterial cells, a portion of 84P2A9 was fused to the Glutathione S-transferase (GST) gene by cloning into pGEX-6P-1 (Amersham Pharmacia Biotech, NJ). All constructs were made to generate recombinant 84P2A9 protein sequences with GST fused at the N-terminus and a six histidine epitope at the C-terminus. The six histidine epitope tag was generated by adding the histidine codons to the cloning primer at the 3' end of the ORF. A PreScission™ recognition site permits cleavage of the GST tag from 84P2A9. The ampicillin resistance gene and pBR322 origin permits selection and maintenance of the plasmid in *E. coli*. In this construct, a fragment containing amino acids 1 to 151 of 84P2A9 was cloned into pGEX-6P-1.

Additional constructs can be made in pGEX-6P-1 spanning regions of the 84P2A9 protein such as amino acids 1 to 504 and amino acids 151 to 504.

MAMMALIAN CONSTRUCTS

- 5 To express recombinant 84P2A9 in mammalian systems, the full length 84P2A9 cDNA can for example, be cloned into an expression vector that provides a 6His tag at the carboxyl-terminus (pCDNA 3.1 myc-his, Invitrogen). The constructs can be transfected into 293T cells. Transfected 293T cell lysates can be probed with the anti-84P2A9 polyclonal serum described in Example 4 above in a Western blot.
- 10 The 84P2A9 genes can also be subcloned into the retroviral expression vector pSRαMSVtkneo and used to establish 84P2A9 expressing cell lines as follows. The 84P2A9 coding sequence (from translation initiation ATG to the termination codons) is amplified by PCR using ds cDNA template from 84P2A9 cDNA. The PCR product is subcloned into pSRαMSVtkneo via the EcoR1(blunt-ended) and Xba 1 restriction sites
- 15 on the vector and transformed into DH5α competent cells. Colonies are picked to screen for clones with unique internal restriction sites on the cDNA. The positive clone is confirmed by sequencing of the cDNA insert. Retroviruses may thereafter be used for infection and generation of various cell lines using, for example, NIH 3T3, TsuPr1, 293 or rat-1 cells.
- 20 Specific mammalian systems are discussed herein.

Production of Recombinant 84P2A9 using pcDNA3.1/V5-His-TOPO Constructs

- To express 84P2A9 in mammalian cells, the 1512 bp (504 amino acid) 84P2A9 ORF along with perfect translational start Kozak consensus sequence was cloned into
- 25 pcDNA3.1/V5-His-TOPO (Invitrogen, Carlsbad, CA). Protein expression is driven from the cytomegalovirus (CMV) promoter. The recombinant protein has the V5 epitope and six histidines fused to the C-terminus. The pcDNA3.1/V5-His-TOPO vector also contains the bovine growth hormone (BGH) polyadenylation signal and transcription termination sequence to enhance mRNA stability along with the SV40
- 30 origin for episomal replication and simple vector rescue in cell lines expressing the large T antigen. The Neomycin resistance gene allows for selection of mammalian cells

expressing the protein and the ampicillin resistance gene and ColE1 origin permits selection and maintenance of the plasmid in E. coli.

pSRa Constructs

5 To generate mammalian cell lines expressing 84P2A9 constitutively, the 1551 bp (517 amino acid) ORF is being cloned into pSRa constructs. Amphotropic and ecotropic retroviruses are generated by transfection of pSRa constructs into the 293T-10A1 packaging line or co-transfection of pSRa and a helper plasmid (ϕ □) in 293 cells, respectively. The retrovirus can be used to infect a variety of mammalian cell lines, 10 resulting in the integration of the cloned gene, 84P2A9, into the host cell-lines. Protein expression is driven from a long terminal repeat (LTR). The Neomycin resistance gene allows for selection of mammalian cells expressing the protein and the ampicillin resistance gene and ColE1 origin permits selection and maintenance of the plasmid in E. coli. Additional pSRa constructs are being made to produce both N-terminal and C- 15 terminal GFP and myc/6 HIS fusion proteins of the full-length 84P2A9 protein.

Example 6: Production of Recombinant 84P2A9 in a Baculovirus System

To generate a recombinant 84P2A9 protein in a baculovirus expression system, the 84P2A9 cDNA is cloned into the baculovirus transfer vector pBlueBac 4.5 20 (Invitrogen), which provides a His-tag at the N-terminus. Specifically, pBlueBac-84P2A9 is co-transfected with helper plasmid pBac-N-Blue (Invitrogen) into SF9 (Spodoptera frugiperda) insect cells to generate recombinant baculovirus (see Invitrogen instruction manual for details). Baculovirus is then collected from cell supernatant and purified by plaque assay.

25 Recombinant 84P2A9 protein is then generated by infection of HighFive insect cells (InVitrogen) with the purified baculovirus. Recombinant 84P2A9 protein may be detected using anti-84P2A9 antibody. 84P2A9 protein may be purified and used in various cell based assays or as immunogen to generate polyclonal and monoclonal antibodies specific for 84P2A9.

Example 7: Chromosomal Mapping of the 84P2A9 Gene

The chromosomal localization of 84P2A9 was determined using the GeneBridge4 radiation hybrid panel (Walter et al., 1994, Nat. Genetics 7:22) (Research Genetics, Huntsville Al). The following PCR primers were used to localize 84P2A9:

5

84P2A9.1 gacttcaactgatgcgtggtaggt (SEQ ID NO: 17)

84P2A9.2 gtcatactttccgatgcttgct (SEQ ID NO: 18)

The resulting mapping vector for the 93 radiation hybrid panel DNAs was:

10 0000100011001011001000001100010010010000100010100110001000000010010010010
10000000100000010000. This vector and the mapping program at <http://www.genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl> placed 84P2A9 on chromosome 1q32.3 (D1S1602- D1S217).

15 **Example 8: Identification of Potential Signal Transduction Pathways**

To determine whether 84P2A9 directly or indirectly activates known signal transduction pathways in cells, luciferase (luc) based transcriptional reporter assays are carried out in cells expressing 84P2A9. These transcriptional reporters contain consensus binding sites for known transcription factors that lie downstream of well 20 characterized signal transduction pathways. The reporters and examples of there associated transcription factors, signal transduction pathways, and activation stimuli are listed below.

1. NFkB-luc, NFkB/Rel; Ik-kinase/SAPK; growth/apoptosis/stress
2. SRE-luc, SRF/TCF/ELK1; MAPK/SAPK; growth/differentiation
3. AP-1-luc, FOS/JUN; MAPK/SAPK/PKC; growth/apoptosis/stress
4. ARE-luc, androgen receptor; steroids/MAPK; growth/differentiation/apoptosis
5. p53-luc, p53; SAPK; growth/differentiation/apoptosis
6. CRE-luc, CREB/ATF2; PKA/p38; growth/apoptosis/stress

30

84P2A9-mediated effects may be assayed in cells showing mRNA expression. Luciferase reporter plasmids may be introduced by lipid mediated transfection (TFX-50, Promega). Luciferase activity, an indicator of relative transcriptional activity, is measured by incubation of cells extracts with luciferin substrate and luminescence of the reaction is monitored in a luminometer.

Example 9: Generation of 84P2A9 Monoclonal Antibodies

To generate MAbs to 84P2A9, typically Balb C mice are immunized intraperitoneally with about 10-50 µg of protein immunogen mixed in complete Freund's adjuvant. Protein immunogens include bacterial and baculovirus produced recombinant 84P2A9 proteins and mammalian expressed human IgG FC fusion proteins. Mice are then subsequently immunized every 2-4 weeks with 10-50 µg of antigen mixed in Freund's incomplete adjuvant. Alternatively, Ribi adjuvant is used for initial immunizations. In addition, a DNA-based immunization protocol is used in which a mammalian expression vector such as pCDNA 3.1 encoding the 84P2A9 cDNA alone or as an IgG FC fusion is used to immunize mice by direct injection of the plasmid DNA. This protocol is used alone and in combination with protein immunogens. Test bleeds are taken 7-10 following immunization to monitor titer and specificity of the immune response. Once appropriate reactivity and specificity is obtained as determined by ELISA, Western blotting, and immunoprecipitation analyses, fusion and hybridoma generation is then carried with established procedures well known in the art (Harlow and Lane, 1988).

In a typical specific protocol, a glutathione-S-transferase (GST) fusion protein encompassing an 84P2A9 protein is synthesized and used as immunogen. Balb C mice are initially immunized intraperitoneally with 10-50 µg of the GST-84P2A9 fusion protein mixed in complete Freund's adjuvant. Mice are subsequently immunized every 2 weeks with 10-50 µg of GST-84P2A9 protein mixed in Freund's incomplete adjuvant for a total of 3 immunizations. Reactivity of serum from immunized mice to full length 84P2A9 protein is monitored by ELISA using a partially purified preparation of HIS-tagged 84P2A9 protein expressed from 293T cells (Example 5). Mice showing the

strongest reactivity are rested for 3 weeks and given a final injection of fusion protein in PBS and then sacrificed 4 days later. The spleens of the sacrificed mice are then harvested and fused to SPO/2 myeloma cells using standard procedures (Harlow and Lane, 1988). Supernatants from growth wells following HAT selection are screened by 5 ELISA and Western blot to identify 84P2A9 specific antibody producing clones.

The binding affinity of an 84P2A9 monoclonal antibody may be determined using standard technology. Affinity measurements quantify the strength of antibody to epitope binding and may be used to help define which 84P2A9 monoclonal antibodies are preferred for diagnostic or therapeutic use. The BIACore system (Uppsala, Sweden) 10 is a preferred method for determining binding affinity. The BIACore system uses surface plasmon resonance (SPR, Welford K. 1991, Opt. Quant. Elect. 23:1; Morton and Myszka, 1998, Methods in Enzymology 295: 268) to monitor biomolecular interactions in real time. BIACore analysis conveniently generates association rate constants, dissociation rate constants, equilibrium dissociation constants, and affinity constants.

15

Example 10: In Vitro Assays of 84P2A9 Function

The expression of 84P2A9 in prostate cancer provides evidence that this gene has a functional role in tumor progression. It is possible that 84P2A9 functions as a transcription factor involved in activating genes involved in tumorigenesis or repressing 20 genes that block tumorigenesis. 84P2A9 function can be assessed in mammalian cells using in vitro approaches. For mammalian expression, 84P2A9 can be cloned into a number of appropriate vectors, including pcDNA 3.1 myc-His-tag (Example 5) and the retroviral vector pSRαtkneo (Muller et al., 1991, MCB 11:1785). Using such expression vectors, 84P2A9 can be expressed in several cell lines, including NIH 3T3, rat-1, TsuPr1 25 and 293T. Expression of 84P2A9 can be monitored using anti-84P2A9 antibodies (see Examples 4 and 9).

Mammalian cell lines expressing 84P2A9 can be tested in several in vitro and in vivo assays, including cell proliferation in tissue culture, activation of apoptotic signals, tumor formation in SCID mice, and in vitro invasion using a membrane invasion culture 30 system (MICS) (Welch et al., Int. J. Cancer 43: 449-457). 84P2A9 cell phenotype is

compared to the phenotype of cells that lack expression of 84P2A9. The transcriptional effect of 84P2A9 can be tested by evaluating the effect of 84P2A9 on gene expression using gene arrays (Clontech) and transcriptional reporter assays (Stratagene).

- Cell lines expressing 84P2A9 can also be assayed for alteration of invasive and migratory properties by measuring passage of cells through a matrigel coated porous membrane chamber (Becton Dickinson). Passage of cells through the membrane to the opposite side is monitored using a fluorescent assay (Becton Dickinson Technical Bulletin #428) using calcein-Am (Molecular Probes) loaded indicator cells. Cell lines analyzed include parental and 84P2A9 overexpressing PC3, NIH 3T3 and LNCaP cells.
- 5 To determine whether 84P2A9-expressing cells have chemoattractant properties, indicator cells are monitored for passage through the porous membrane toward a gradient of 84P2A9 conditioned media compared to control media. This assay may also be used to qualify and quantify specific neutralization of the 84P2A9 induced effect by candidate cancer therapeutic compositions.
- 10 The function of 84P2A9 can be evaluated using anti-sense RNA technology coupled to the various functional assays described above, e.g. growth, invasion and migration. Anti-sense RNA oligonucleotides can be introduced into 84P2A9 expressing cells, thereby preventing the expression of 84P2A9. Control and anti-sense containing cells can be analyzed for proliferation, invasion, migration, apoptotic and transcriptional
- 15 potential. The local as well as systemic effect of the loss of 84P2A9 expression can be evaluated.

Example 11: In Vivo Assay for 84P2A9 Tumor Growth Promotion

- The effect of the 84P2A9 protein on tumor cell growth may be evaluated in vivo by gene overexpression in tumor-bearing mice. For example, SCID mice can be injected SQ on each flank with 1×10^6 of either PC3, TSUPR1, or DU145 cells containing tkNeo empty vector or 84P2A9. At least two strategies may be used: (1) Constitutive 84P2A9 expression under regulation of a promoter such as a constitutive promoter obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma
- 25

virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40), or from heterologous mammalian promoters, *e.g.*, the actin promoter or an immunoglobulin promoter, provided such promoters are compatible with the host cell systems, and (2) Regulated expression under control of an inducible vector system, such as ecdysone, tet, 5 etc., provided such promoters are compatible with the host cell systems. Tumor volume is then monitored at the appearance of palpable tumors and followed over time to determine if 84P2A9 expressing cells grow at a faster rate and whether tumors produced by 84P2A9-expressing cells demonstrate characteristics of altered aggressiveness (*e.g.* enhanced metastasis, vascularization, reduced responsiveness to chemotherapeutic 10 drugs). Additionally, mice may be implanted with 1×10^5 of the same cells orthotopically to determine if 84P2A9 has an effect on local growth in the prostate or on the ability of the cells to metastasize, specifically to lungs, lymph nodes, and bone marrow.

The assay is also useful to determine the 84P2A9 inhibitory effect of candidate therapeutic compositions, such as for example, 84P2A9 intrabodies, 84P2A9 antisense 15 molecules and ribozymes.

Example 12: Western Analysis of 84P2A9 Expression in Subcellular Fractions

Sequence analysis of 84P2A9 revealed the presence of nuclear localization signal. The cellular location of 84P2A9 can be assessed using subcellular fractionation 20 techniques widely used in cellular biology (Storrie B, et al. Methods Enzymol. 1990;182:203-25). Prostate or testis cell lines can be separated into nuclear, cytosolic and membrane fractions. The expression of 84P2A9 in the different fractions can be tested using Western blotting techniques.

Alternatively, to determine the subcellular localization of 84P2A9, 293T cells can 25 be transfected with an expression vector encoding HIS-tagged 84P2A9 (PCDNA 3.1 MYC/HIS, Invitrogen). The transfected cells can be harvested and subjected to a differential subcellular fractionation protocol as previously described (Pemberton, P.A. et al, 1997, J of Histochemistry and Cytochemistry, 45:1697-1706.) This protocol separates the cell into fractions enriched for nuclei, heavy membranes (lysosomes, peroxisomes,

and mitochondria), light membranes (plasma membrane and endoplasmic reticulum), and soluble proteins.

5 Throughout this application, various publications are referenced (within parentheses for example). The disclosures of these publications are hereby incorporated by reference herein in their entireties.

10 The present invention is not to be limited in scope by the embodiments disclosed herein, which are intended as single illustrations of individual aspects of the invention, and any that are functionally equivalent are within the scope of the invention. Various modifications to the models and methods of the invention, in addition to those described herein, will become apparent to those skilled in the art from the foregoing description and teachings, and are similarly intended to fall within the scope of the invention. Such 15 modifications or other embodiments can be practiced without departing from the true scope and spirit of the invention.

TABLES

TABLE 1: predicted binding of peptides from 84P2A9 proteins to the human MHC class I molecule HLA-A2

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of half time of disassociation)
1	300	SILTGSFPL (SEQ ID NO: 19)	63.04
2	449	RMLQNMGWT (SEQ ID NO: 20)	33.75
3	4	LVHDLVSAI (SEQ ID NO: 21)	29.97
4	238	SLSSTDAGL (SEQ ID NO: 22)	21.36
5	198	KIQDEGVVL (SEQ ID NO: 23)	17.28
6	433	FVGENAQPI (SEQ ID NO: 24)	17.22
7	301	ILTGSFPLM (SEQ ID NO: 25)	16.05
8	218	KMECEEQKV (SEQ ID NO: 26)	11.25
9	480	KGLGLGFPL (SEQ ID NO: 27)	10.47
10	461	GLGRDGKGI (SEQ ID NO: 28)	10.43

TABLES 3-16 provide additional analyses of the predicted binding of peptides from 84P2A9 proteins to various HLA molecules.

$$\frac{\partial \ln P_{\text{obs}}}{\partial \theta} = \frac{\partial \ln P_{\text{obs}}}{\partial \theta'} \frac{\partial \theta'}{\partial \theta} = \frac{\partial \ln P_{\text{obs}}}{\partial \theta'} \frac{\partial \theta'}{\partial \theta}$$

TABLE 2: AMINO ACID SUBSTITUTION MATRIX

Adapted from the GCG Software 9.0 BLOSUM62 amino acid substitution matrix (block substitution matrix). The higher the value, the more likely a substitution is found in related, natural proteins.

	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
4	0	-2	-1	-2	0	-2	-1	-1	-1	-2	-1	-1	-1	-1	1	0	0	-3	-2	A
9	-3	-4	-2	-3	-3	-1	-3	-1	-1	-3	-3	-3	-3	-1	-1	-1	-2	-2	C	
6	2	-3	-1	-1	-3	-1	-4	-3	1	-1	0	-2	0	-1	-3	-4	-3	D		
5	-3	-2	0	-3	1	-3	-2	0	-1	2	0	0	-1	-2	-3	-2	E			
6	-3	-1	0	-3	0	0	-3	-4	-3	-3	-2	-2	-1	1	1	3	F			
6	-2	-4	-2	-4	-3	0	-2	-2	-2	0	-2	-2	-2	-2	-2	-3	-2	G		
8	-3	-1	-3	-2	1	-2	0	0	-1	-2	-3	-2	-2	-2	-2	-2	H			
4	-3	2	1	-3	-3	-3	-3	-2	-1	3	-3	-1	-1	-1	-1	-1	I			
5	-2	-1	0	-1	1	2	0	-1	-2	-2	-3	-2	K							
4	2	-3	-2	-2	-2	-2	-2	-2	-1	1	-2	-1	L							
5	-2	-2	0	-1	-1	-1	-1	-1	-1	1	-1	-1	M							
6	-2	0	0	1	0	-3	-4	-2	N											
7	-1	-2	-1	-1	-2	-4	-3	P												
5	1	0	-1	-2	-2	-1	Q													
5	-1	-1	-3	-3	-2	R														
4	1	-2	-3	-2	S															
5	0	-2	-2	T																
4	-3	-1	V																	
11	2	W																		
7	Y																			

HLA peptide motif search results

User Parameters and Scoring Information	
method selected to limit number of results	explicit number
number of results requested	30
HLA molecule type selected	A1
length selected for subsequences to be scored	9
echoing mode selected for input sequence	Y
echoing format	numbered lines
length of user's input peptide sequence	504
number of subsequence scores calculated	496
number of top-scoring subsequences reported back in scoring output table	30

TABLE 3A

Scoring Results

Rank	Start Position	Subsequent Residue Listing	Score (Estimate of Half-life of Disassociation of a Molecule Containing This Subsequence)
1	71	SLEEPSKDY	45.000
2	469	ISEPPIQAMQ	27.000
3	283	KEDPTELDK	25.000
4	15	SSEQQARGGF	13.500
5	23	FAETGDHSR	9.000
6	441	ILENNIGNR	9.000
7	241	STDAGLFTN	6.250
8	72	LEEPSKDYR	4.500
9	233	ESDSSSLSS	3.750
10	92	DSDQMLVVA	3.750
11	157	MTPQPPGCR	2.500
12	413	TGDIKRRRK	2.500
13	256	DDEQSDWFY	2.250
14	373	RTEHDHQHL	2.250
15	309	MSHPSRRGF	1.500
16	207	ESEETNQTN	1.350
17	231	MSESDSSSL	1.350
18	64	LSEGSDSL	1.350
19	456	WTPCGSLGR	1.250
20	375	EHDHQHQLR	1.250
21	293	VDPDVVFESI	1.250
22	93	SDDQMLVAK	1.000
23	494	ATTTPNAGK	1.000
24	208	SEETNQTNK	0.900
25	205	VLESEETNQ	0.900
26	79		0.900
27	11	ALEESSEQA	0.900
28	226	VSDELMSES	0.750
29	31	RSISCPPLKR	0.750
30	90	HSDSDDQML	0.750

**Echoed User Peptide Sequence
(length = 504 residues)**

1 MEELVHDLVS ALEESSEQAR GGFAETGDHS RSISCPLKRQ ARKRRGRKRR
 51 SYNVHHWET GHCLSEGSQS SLEEPSKDYR ENHINNNKKDDI SDSDDDQMLVA
 101 KRRPSSNLNN NVRGKRLPLWH ESDFAVDNVG NRTLRRRKV KRMADVLPQD
 151 ISGNKRTMTQF PEGCRDQDMO SDRAYQYQEF TKNKVKKRKL KIIHQGPQIQ
 201 DEGVWLSEEP TNQTNKDKMEE CEEQKVSDMS MSESODSSSL STDAGLETFND
 251 EGROGGDDEQF DWFYKEESGG ACCGITGVWPW WEKEDPTELD KNVPDPVFES
 301 ILTGSFLPLMS HPSRRGEQAR LSRLHGMSST NIKKSGGTPT SMVPIPVG
 351 NKRWHVHESPD SHHHDHWSP GARTEDHQH LLRDNRRAERG HKKNCSVRTA
 401 SRQTSMLGLS LCTGDIKRRR KAAPLPFGTT AGFVGENAQF ILENNIGNRM
 451 LQNMGMWTPGS GLGRDGKGIS EPIQAMQRPK GLGLGFPLPK STSATTPNA
 501 GKSA

TABLE 3B

HLA peptide motif search results

User Parameters and Scoring Information	
method selected to limit number of results	explicit number
number of results requested	30
HLA molecule type selected	A1
length selected for subsequences to be scored	10
echoing mode selected for input sequence	Y
echoing format	numbered lines
length of user's input peptide sequence	504
number of subsequence scores calculated	495
number of top-scoring subsequences reported back in scoring output table	30

TABLE 4A

Scoring Results			
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	469	ISEP <small>i</small> QAMOR	675.000
2	92	DSDDqMLVAK	30.000
3	207	ESEEtNQTNK	27.000
4	168	DMDSdRAYQY	25.000
5	11	ALEEsSEQAR	9.000
6	71	SLEEP <small>p</small> SKDYL	9.000
7	282	EKEOpTELDK	4.500
8	166	DQDMdSDRAY	3.750
9	90	HSDSdDQMLV	3.750
10	177	YQEFTKNKVK	2.700
11	144	AVDLP <small>p</small> QDISN	2.500
12	373	RTEHdHQHQLL	2.250
13	33	ISCP1KROAR	1.500
14	231	MSESDSSSLs	1.350
15	15	SSEQaRGGFA	1.350
16	254	CGDDeQSDWF	1.250
17	255	GDDEqSDWFY	1.250
18	293	VPDFvFESIL	1.250
19	173	RAYQyQEFTK	1.000
20	481	GLGLgFFPLPK	1.000
21	205	VLESeETNQT	0.900
22	79	YRENhNNNKK	0.900
23	441	ILENnIGNRM	0.900
24	23	FAETgDHRS	0.900
25	121	ESDFAVDNVG	0.750
26	233		0.750
27	409	GSLCtGDIKR	0.750
28	259	QSDWFYEKES	0.750
29	70	SSLEePSKDY	0.750
30	67	GSDSsLEEPS	0.750

Echoed User Peptide Sequence (length = 504 residues)

1 MEELVHDLVS ALEESSEQAR GGFATGQHDS RS1SCPLKRQ ARKRRGRKRR
 51 SYNVVHFPEW GHLCLSEGSDS SLEEPskDYL ENHNNNNKKD SDSDQDMLV
 101 KRRPSNLNN NVRGRPLWH ESDFAVQDVG NRTLRRRKV KRMADVLDQD
 151 ISNRKTMTQP PEGCDRQDMO SDRAYQyQEFTK TKVKKKRL KIIRQGPKIQ
 201 DEGVVLESEE TNQTKDRME CEEQKVSDEL MSESDSSSLs STDAGLFTND
 251 EGRRDDEQES DWYEYKESPPG ACGITGVVFW WEKEPDPTED KNVPDPVFES
 301 ILTGSFPPLMS HPSRRGFQAR LSRLHGMSK NIKKSGGTP SMVPIPGPVG
 351 NKRVMHFSPP SHHCDHWFSP GARTEHDQHO LLRDNRAERG HKKNCSVRTA
 401 SROTSMHLGS LCTGDIKRR KAALPFGPTT AGFVGENAQI ILENNIGNRM
 451 LQNMGWTFGS GLGRDGKGIS EPIQAMQRPK GLGLGFFPLPK STSATTPNA
 501 GKSa

TABLE 4B

HLA peptide motif search results

User Parameters and Scoring Information	
method selected to limit number of results	explicit number
number of results requested	30
HLA molecule type selected	A 0201
length selected for subsequences to be scored	9
echoing mode selected for input sequence	Y
echoing format	numbered lines
length of user's input peptide sequence	504
number of subsequence scores calculated	496
number of top-scoring subsequences reported back in scoring output table	30

TABLE 5A

Scoring Results			
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	300	SILTGSPPL	63.035
2	449	RMLQNMCGWT	32.748
3	4	LVHDLVSLA	29.965
4	238	SLSSTDAGL	21.362
5	198	KIQDEGVVL	17.282
6	433	FVGENAQPI	17.217
7	301	ILTGSFFLM	16.047
8	218	KMECEEQKV	11.252
9	480	KGLGLGFPL	10.474
10	461	GLGRDGKGI	10.433
11	341	SMVRIPGPV	6.530
12	468	GISEPIQAM	6.442
13	405	SMHLGSLCT	5.382
14	191	KIIRQGPKI	5.021
15	117	PLWHESDFA	2.445
16	177	YQEFTKNKV	2.076
17	454	MGWTPGSGL	1.968
18	156	TMTQPPPEC	1.758
19	374	TEHDQHOLL	1.703
20	52	YNVHHWPET	1.678
21	474	QAMQRPKGL	1.098
22	240	SSTDAGLFT	1.097
23	438	AQPILENNI	1.058
24	269	GGACGITGV	1.044
25	143	MAVDLPQDI	1.010
26	206	LESEETNQT	1.010
27	173	RAYQYQEFF	0.893
28	3	ELVHDLVSA	0.837
29	132	RTLRRRKV	0.715
30	266	KESGGACGT	0.710

Echoed User Peptide Sequence (length = 504 residues)

1 MEELVHDLVS ALEESSEQAR GGFATGDHS RSISCPPLKRQ ARKRRGRKRR
 51 SYNVHFWET GHCLSEGSDS SLEEPSKDYL ENHNNNNKKDH SDSDDQMLVA
 101 KRRPSSNLNN NVRGKRPPLWH ESDFAVDNVG NRTLRRRKV KRMADVLPQD
 151 ISNKRTMTQP PEGCHQDMD SDRAYQYQEF TKNKVKKKRL KIIRQGPKIQ
 201 DEGVQDVEE TNQTNKDKME CEEQKRVSDL MSESQSSLLS STDAGLFIND
 251 EGRQQDDEQS DWFYKEESCG AGCIGTVWPV WEKEDPTELD KNVPDPVVEES
 301 ILTGSFFPLMS HPSRSRGFQAR LSRLIGMSKK NIKKSGGTLD SMVPIPGPVG
 351 NKRMVHFESPD SHHHDWHPSP GARTEHDQHQ LLRDNRAERG HKKNCVRIIA
 401 SRQTSMLHGS LCTGDIKRRR KAALPLPGPTT AGFVGENAQPI ILENNIGNRM
 451 LQNGWTPGSS GLGRDGKGIS EPIQAMQRPK GLGLGFPPLPK STSATTTPNA
 501 GKSA

TABLE 5B

HLA peptide motif search results

User Parameters and Scoring Information	
method selected to limit number of results	explicit number
number of results requested	30
HLA molecule type selected	A_0201
length selected for subsequences to be scored	10
echoing mode selected for input sequence	Y
echoing format	numbered lines
length of user's input peptide sequence	504
number of subsequence scores calculated	495
number of top-scoring subsequences reported back in scoring output table	30

TABLE 6A

Scoring Results			
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	230	LMSEgSDSSL	107.536
2	63	CLSEgSDSSL	87.586
3	117	PLWHeSDFAV	73.661
4	453	NNGWT-PGSGL	15.428
5	475	AMQRpKGLGL	15.428
6	433	FVGEaAQPIL	14.454
7	323	RLHGMSSKNI	10.433
8	142	RMAVdLPQDI	7.535
9	483	GLGFpLPKST	7.452
10	300	SILTgSFPLM	4.802
11	3	ELVHdLVGAL	3.685
12	473	IQAMqrPKCL	3.682
13	292	NVPDpVFESI	3.485
14	124	FAVDrVGVRT	1.952
15	334	KSGGCPTSMV	1.589
16	445	NIGNiMLQNM	1.571
17	315	RGFOaRLSRL	1.187
18	268	SGGAcGITGV	1.044
19	288	ELDKnVPDPV	1.022
20	486	FPLPkSTSAT	0.828
21	205	VLESeETNOT	0.811
22	402	ROTSmHLGSL	0.648
23	425	LPGptTAGFV	0.552
24	441	ILENnIGNRM	0.541
25	237	SSLSsTDAGL	0.516
26	10	SALLeSSSEQA	0.513
27	212	NQTNKDKMEC	0.504
28	301	ILTGSFPLMS	0.481
29	239	LSSTGAGLFT	0.455
30	103	RPSSsLNNNV	0.454

**Echoed User Peptide Sequence
(length = 504 residues)**

1 MEELVHDLVS ALEESSEQAR GGFATGDKHS RSISCPPLKRQ ARKRRGRKRR
 51 SYNVRHPWET GHCLSEGGDS SLEEPSKDYR ENHHNNKKDH SDSDQQMLVA
 101 KRPSSNLLN NVRGKPLWH ESDFAVNDVG NRTLRRRRKV KRMAVDLPOD
 151 ISNKRTMTQP PEGCRDQMDM SDRAYQYQEF TKNVKVKKRL KIIHQGPQIQ
 201 DEGVYLESEE TNQTNKDKME CEEQKVSDEL MSSESDDSSLS STDAGLFTND
 251 EGRRQDDEQS DWFYKEESGG ACGITGVVFW KEWEDPTELD KNVDPDVIES
 301 ILTGSPFLMS HPSRQGFQAR LSRLHGMSSK NIKKSSGGTPT SMVPIPGPGV
 351 NKRVMWHPSPD SHHHHDWFSP GARTEHDHQ LLRDNRAERG HKKNCSVRTA
 401 SRQTSMHIGS LCTGDIKRRR KAALPLPGFT AGFVGENAQP ILENNIGNRM
 451 LONMGWTGGS GLGRDGKGIS EPIQAMQRPK GLGLGFPLPK STSATTTPNA
 501 GKSA

TABLE 6B

HLA peptide motif search results

User Parameters and Scoring Information	
method selected to limit number of results	explicit number
number of results requested	30
HLA molecule type selected	A3
length selected for subsequences to be scored	9
echoing mode selected for input sequence	Y
echoing format	numbered lines
length of user's input peptide sequence	504
number of subsequence scores calculated	496
number of top-scoring subsequences reported back in scoring output table	30

TABLE 7A

Scoring Results			
Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half-time of Disassociation of a Molecule Containing This Subsequence)
1	326	GMSSKNIKK	120.000
2	245	GLFTNDEGR	60.000
3	133	TIRRKKVK	10.000
4	146	DLPQDINSK	9.000
5	410	SLCTGDIKR	8.000
6	107	NLNNNNVRGK	6.000
7	258	EQSDWFWYK	4.860
8	71	SLEEPSSKDY	4.500
9	381	LLRDNRAER	4.000
10	441	I LENNIGNR	1.800
11	494	ATTPPNACK	1.500
12	301	ILTGSFPLM	0.900
13	461	GLGRDGKG I	0.900
14	128	NVGNRTLRR	0.800
15	238	SLSSTDAGL	0.600
16	307	PLMSHPSRR	0.600
17	456	WTPGSGLGR	0.600
18	218	KMECEEQKV	0.600
19	283	KEDPTELDK	0.540
20	409	GSLCTGDIK	0.450
21	273	GITGVVPWW	0.405
22	344	PIPGPVGNK	0.405
23	184	KVKKRKLKI	0.360
24	156	TMTQPPUGC	0.300
25	11	ALEESSEQA	0.300
26	180	FTKNKVKKR	0.300
27	35	CPLKRQARK	0.300
28	459	GSGLGRDGK	0.300
29	191	KIIRQGPKI	0.270
30	483	GLGFPLPKS	0.270

Echoed User Peptide Sequence
(length = 504 residues)

1 MEELVHDLV S ALEESSEQAR GGFATGDDH RSISCP LKQ ARKRRGRKRR
 51 SVNVHPNET GHCLSEGS DSDS SLEEP SKD Y ENHN NKKD SDSD CMVA
 101 KAR PSSN LN NN VNR GKR PLWH ESD FAV DVNG NRT LRRRKV KRM AVD LPQD
 151 ISNK RTMT QP PEG CRD QMD SD RAY QY QEF TKN KVKK RL KI RQ GP KI Q
 201 DEGV VLE SEE TN QTN KDKM E CEE QKV S DEL MS ESD SSS L S STD AGLE TN D
 251 EGR QGD DEQS DW FYKE ESGG ACG ITG VVPW W ERED PT ELD KN VP DP VF ES
 301 ILT GS FPL M HPS RRG FQ AR SLR LHG MSS K NI K SG GT P SM VP IGP PG V
 351 NK RV HF QSP SHHH DHW FSP GAR TEHD HQO LL RD NR AERG HK KN CS VR TA
 401 SRO TS MH GS L CT GD IK RR KA AP L PG TT AG FV GE NA QP I L ENN I GR NM
 451 LQN MG WT PGS GL GR DG KG IS E PI QAM QR PK GL GL GF PL PK ST S ATT PNA
 501 GKS A

TABLE 7B

HLA peptide motif search results

User Parameters and Scoring Information	
method selected to limit number of results	explicit number
number of results requested	30
HLA molecule type selected	A3
length selected for subsequences to be scored	10
echoing mode selected for input sequence	Y
echoing format	numbered lines
length of user's input peptide sequence	504
number of subsequence scores calculated	495
number of top-scoring subsequences reported back in scoring output table	30

TABLE 8A

8

Scoring Results

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	481	GLGLgFPLPK	360.00
2	189	KLKII1RQGPK	18.000
3	71	SLEEpSKDYL	6.000
4	11	ALEEeSSEQAR	6.000
5	380	QLLRGNRAER	6.000
6	175	YQQQeFTKNNK	4.500
7	274	ITGVvPPWEEK	4.500
8	133	TLLRrRKVKR	4.000
9	168	DMDSDRAYQY	3.600
10	173	RAYQyQEFTK	3.000
11	410	SLCTgDIKRR	3.000
12	156	TMTQpPEGCR	1.800
13	146	DLpQDISNKR	1.800
14	107	NLNNNnVRGKR	1.800
15	475	AMQRpKGLCL	1.200
16	63	CLSeqSDSSL	0.900
17	453	MMGWtPGSGL	0.900
18	230	LMSeqDSSSL	0.900
19	3	ELVHdILVSAL	0.810
20	132	FTLRLRRRKV	0.750
21	180	FTKNNKVKKRK	0.750
22	343	VPIPgPVGNK	0.608
23	238	SLSStDAGLF	0.600
24	142	RMAVGLPQDI	0.600
25	257	DEQSdWFYEK	0.486
26	323	RLHGmSSKNI	0.450
27	301	ILTGSFFPLMS	0.360
28	117	PLWHeSDFAV	0.300
29	493	SATTtPNACK	0.300
30	177	YQEFTKNVKV	0.300

Echoed User Peptide Sequence
(length = 504 residues)

1 MEELVHDIVS ALEESSEQAR GGFATGDHS RSICSPILKQ ARKRRGRKRR
 51 SYNVHHHPWET GHCLSeGSDS SLEEPSKDYL ENHNNNNKKDH SDSDDQMLVA
 101 KRRPSSNLNN NVRGKRFLWES DSEFADVONVG NRTLRRRRRV KRMAVDLPOD
 151 ISNKRTMTQF PEGCRDQDM SDRAYQYQEF TKNVKKKRKL KIIRQGPQIQ
 201 DEGVVLESEE TNQTNKDKMEE CEEQKVSDLS MSSESDDSSLS STDAGLFTND
 251 EGRQGDDEQS DWFYEKESGG ACCITGVVWP WEKEADPTELD KNVFDPVFES
 301 ILTGSPFLMS HPSPRRGFQAR LSRLHGMSK NIKKSGGTPT SMVPIPQPGVG
 351 NKRVMVHPSPD SHHHHDWFSP GARTEHDHQ LLLRDNRRAERG HKKNCSVRTA
 401 SRQTSNMHLGS LCTGDIKKRR KAAPLPGFTT AGFVGENAQP ILENNIGNRM
 451 LQNMGWTPGS GLGRDGKGIS EPIQAMQRPK GLGLGFPLPK STSATTTPNA
 501 GKSA

TABLE 8B

HLA peptide motif search results

User Parameters and Scoring Information	
method selected to limit number of results	explicit number
number of results requested	30
HLA molecule type selected	A_1101
length selected for subsequences to be scored	9
echoing mode selected for input sequence	Y
echoing format	numbered lines
length of user's input peptide sequence	504
number of subsequence scores calculated	496
number of top-scoring subsequences reported back in scoring output table	30

TABLE 9A

Scoring Results

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	326	GMSSKNIKR	2.400
2	174	A Y Q Y Q E F T K	1.200
3	494	A T T T P N A G K	1.000
4	128	N V G N R T L R R	0.800
5	245	G L F T N D E G R	0.480
6	456	W T P G S G L G R	0.400
7	258	E Q S D W F Y E K	0.360
8	283	K E D P T E L D K	0.360
9	35	C P L K R Q A R K	0.300
10	133	T L R R R R K V K	0.200
11	176	Q Y Q E F T K N K	0.200
12	157	M T Q P P E G C R	0.200
13	40	Q A R K R R G R K	0.200
14	80	R E H H N N N K K	0.180
15	410	S L C T G D I K R	0.160
16	210	E T N Q T N K D K	0.150
17	146	D L P Q D I S N K	0.120
18	184	K V K K R K L K I	0.120
19	180	F T K N K V K K R	0.100
20	409	G S L C T G D I K	0.090
21	381	L L R D N R A E R	0.080
22	441	I L E N N I G R N S	0.080
23	482	L C L G F P L P K	0.060
24	459	G S G I L G R D G K	0.060
25	275	T G V V P W W E K	0.060
26	139	K V K R M A V D L	0.060
27	208	S E E T N Q T N K	0.060
28	306	F P L M S H P S R	0.060
29	178	Q E F T K N K V K	0.060
30	179	E E T K N K V K K	0.060

Echoed User Peptide Sequence
(length = 504 residues)

1 MEELVHDLVS ALEESSEQAR GGFAETGDHS RSISCPALKRQ ARKKRGRKRR
 51 SYNHVPET GHCLSEGSDS SLEEPSKDYL ENHNNNKKDKI SDSDQDMLVAA
 101 KRRPSSNLNN NVRCKRPLWH ESDFADVNDVG NRTLRRRRKV KRMADVLDLQD
 151 ISNRKTTMQLP EGCRDQMDM SDRAYQYQEF TKNVKKRKL KIIRQQPKIQ
 201 DEGVVLESEE TNQTKNDKME CEEQKVSDEL MSSESDESSLSS STDAGLFTND
 251 EGRQGDDEQS DWFYKEKESGG ACGITGVVPW WEKEDPTELD KNVPDPVFES
 301 ILTGSFPLMS HPSRGGFQAR LSRLHGMSSK NIKKSGGTPT SMVFIPGFVG
 351 NKRVMVFSPD SHHHHDHWFSP GARTEHDHQH LLRDRRAERG KKKNCSVRTA
 401 SRQTSMLGSG LCTGDIKRRR KAAPLPGPTT AGFVGENAQP ILENNIGNRM
 451 LQNNGWTPGS GLGRDGKGIS EPIQAMQRPK GLGLGFPLPK STSATTPNA
 501 GKSA

TABLE 9B

HLA peptide motif search results

User Parameters and Scoring Information	
method selected to limit number of results	explicit number
number of results requested	30
HLA molecule type selected	A 1101
length selected for subsequences to be scored	10
echoing mode selected for input sequence	Y
echoing format	numbered lines
length of user's input peptide sequence	504
number of subsequence scores calculated	495
number of top-scoring subsequences reported back in scoring output table	30

TABLE 10A

Scoring Results

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half-Time of Disassociation of a Molecule Containing This Subsequence)
1	173	RAYQYQEFTK	3.600
2	481	GLGLgFPLPK	2.400
3	132	RTLRzRRKV	2.250
4	274	ITGVvPWWEK	2.000
5	39	RQARKRRGRK	1.800
6	189	KLKiiRQGPK	1.200
7	175	YQYQeFTKNK	0.600
8	180	FTKNKVKKRK	0.500
9	177	YQEFTKNKVR	0.300
10	343	VPIPgPVGNK	0.300
11	493	SATTtPNAGE	0.200
12	34	SCPLkRQARK	0.200
13	178	QEFTkNKVKK	0.120
14	78	DYREnHNNNK	0.120
15	380	QLLRdNRAER	0.120
16	22	GFAEtGDHSR	0.120
17	412	CTGDIKRARK	0.100
18	133	TLRRzRKVKR	0.080
19	71	SLEEPskDyR	0.080
20	325	HGMssKNIKK	0.080
21	107	NINNNnVRGKR	0.080
22	11	ALEEsSEQAR	0.080
23	156	TMTQpPEGCR	0.080
24	182	KNKVkKRKLR	0.060
25	216	KDKMeCEEQK	0.060
26	383	RDNRaERGHK	0.060
27	306	FPLMSHPSRR	0.060
28	128	NVGNrTLRRR	0.040
29	111	NVRGkRPLWH	0.040
30	311	HPSRrGEQAR	0.040

Echoed User Peptide Sequence
(length = 504 residues)

1 MELLVHDLVS ALEESSEQAR GGFAETCDHS RSISCPKRO ARKRRGRKRR
 51 SVNVHFWET GHCLSEGSDS SLEEPskDyR ENINNNKKD SDSDDQMLVA
 101 KRRSSNLNN NVRGKRLWES DSDFAVDNVG NRTLRRRKV KRMADVLPQD
 151 ISNRKTMTQP PEGCRDDMD SDRAYQYQEG TKNVKKKRKL KIIRQGPKIQ
 201 DEGVVLESEE TNQTNKDKME CEEQKVSDET MSSEDSSSSL STDAGLPTND
 251 EGRGGDDEQG DWFYEKESGG ACGITGVVPW WEKEDPTELS KNVDPVFES
 301 ILTGSFPPLMS HPSRERGEQAR LSRLGMSKK NIKKSGGTPT SMVEIPGPVG
 351 NKRVMHFSPLD SHHHDHWESP GARTEHDHQH LLRDNRAERG HKKNCSVRTA
 401 SROTSMHLGS LCTGDIKRKK KAALPVGTT AGFVGENAQP ILENNIGNRM
 451 LQNMGWTFGS GLGRDGKGIS EPIQAMQRPK GLGLGFPLPK STSATTPNA
 501 GKSA

TABLE 10B

HLA peptide motif search results

User Parameters and Scoring Information	
method selected to limit number of results	explicit number
number of results requested	30
HLA molecule type selected	A24
length selected for subsequences to be scored	9
echoing mode selected for input sequence	Y
echoing format	numbered lines
length of user's input peptide sequence	504
number of subsequence scores calculated	496
number of top-scoring subsequences reported back in scoring output table	30

TABLE 11A

Scoring Results

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	316	GFQARLSRL	30.000
2	480	KCLGLGFPL	14.400
3	198	KIQDEGVVL	14.400
4	373	RTEHDQHQL	12.000
5	182	KNKVKKRKL	8.800
6	139	KVKRMAVDL	8.000
7	263	FYEKESGGA	7.500
8	78	DYRENHNNN	7.200
9	300	SILTGSSPPL	6.000
10	474	QAMQRPKGL	6.000
11	116	RPLWHESDF	6.000
12	110	NNVRGKRPL	6.000
13	231	MSESDSSSL	6.000
14	434	VGENAQFIL	6.000
15	64	LSEGSDSSL	6.000
16	443	ENNIGNRML	6.000
17	4	LVHDLVSL	5.760
18	29	HRSRISCPPL	5.600
19	56	HFWETGHCL	4.800
20	90	HSDSDQDML	4.800
21	478	RPKGGLGLF	4.800
22	476	MQRPKGLGL	4.800
23	400	ASROTSMHL	4.000
24	454	MGWTPGSGL	4.000
25	238	SLSSTDAGL	4.000
26	403	QTSMHLGSL	4.000
27	191	KIIRQGPKII	3.300
28	349	VGNKRMVHF	3.000
29	15	SSEQQARGGF	3.000
30	143	MAVDLPQDI	2.592

**Echoed User Peptide Sequence
(length = 504 residues)**

MEELVHDLVS ALEESSEQAR GGFAETGDHS RSISCELKQR ARKRRGRKRR
 51 SYNVHRPWET GHLSSEGSDS SLEEPSKDYR ENHHNNNNKDH SDSDDQMLVA
 101 KRRPSNLNN NVRGKRPLWH ESDFAVNVG NRTLRRRKV KRMADVLPQD
 151 ISNKRTMTQP PEGCRQDMD SDRAYQYQEF TKNVKVKKRL KIIRQGPKIQ
 201 DEGVVLESEE TNQTNKDKMCE CEEQKVSDEL MSESDSSSL SSTDAGLFND
 251 EGRRGGDDEQS DWFYEKESGG ACCITGVPPW WEKEDEPTD KNVPDPVFS
 301 ILTGGFPPLMS HPGRRGFQAR LSRLHGMSKK NIKKSGGTPT SMVPIPVG
 351 NKRKVMHFSPLS SHHHHHWFSP CARTEHDHQH LLRDNRRAERG HKKNCSVRTA
 401 SRQTSMHLGS LCTGDIKRRR KAAPLGPTT AGFVGNAQP ILENNIGNRM
 451 LQNNGWTPGS GLGRDGKGIS EPIQAMQRPK GLGLGFPLFK STSATTTPNA
 501 GKSA

TABLE 11B

HLA peptide motif search results

User Parameters and Scoring Information	
method selected to limit number of results	explicit number
number of results requested	30
HLA molecule type selected	A24
length selected for subsequences to be scored	10
echoing mode selected for input sequence	Y
echoing format	numbered lines
length of user's input peptide sequence	304
number of subsequence scores calculated	495
number of top-scoring subsequences reported back in scoring output table	30

TABLE 12A

Scoring Results

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	297	VFESILTGSF	18.000
2	373	RTEHQHQHQL	14.400
3	176	QYQEITTKNKV	11.880
4	174	AYQYqEFTKN	9.900
5	432	GFGVGENAQPI	9.000
6	51	SYNVhHPWET	8.250
7	402	RQTSMhHLGSL	8.000
8	315	RGFQqRLSRL	8.000
9	263	FYEKeGGAC	7.500
10	3	ELVHdLVSAL	7.200
11	280	WWEKedPTEL	6.600
12	237	SSLSsTDAGL	6.000
13	299	ESILTgGSFPL	6.000
14	475	AMQRpKGLGL	6.000
15	109	NNNvGKRPL	6.000
16	230	LMSeSDSSL	4.800
17	293	VPDPvFESIL	4.800
18	433	FVGEnAQPIL	4.800
19	63	CLSEgSDSSL	4.800
20	125	AVDNvGNRTL	4.000
21	99	VAKRrPSSNL	4.000
22	473	IQAMqRPKGL	4.000
23	453	NMGWEPGSGL	4.000
24	399	TASRqTSMHL	4.000
25	292	NVPDpVFESI	3.024
26	142	RMAVdLPQDI	2.880
27	437	NAQPILENNI	2.592
28	254	QGDDeQSDWF	2.400
29	14	ESSEqARGGF	2.400
30	323	RLHCmSSKNI	2.000

**Echoed User Peptide Sequence
(length = 504 residues)**

```

1      MEELVHDLVS ALEESSEQAR CGGFAETGDHS RSISCPKLKRQ ARKRRGRKRR
51     SYNVhHPWET GHCLSEGSDS SLEEPSKDYR ENHNNNKKDDI SDSDDDQMLVA
101    KRRPSNLNN NVRGKRPLW HESDFAVDNVG NRTLRRRKV KRMAVQLPQD
151    ISNRKRTMTQP PEGCRDQDMD SDRAYQYQEF TKNVKKKRL KIIROQGPKIQ
201    DEGVVLESEE TNQTNKDKME CEEQKVSDEL MSSEDSSSSLS STDAGLFTND
251    EGQQGDEQS DWFYKEESGG AGCGITGVVFW WEKEDPTELD KNVBDPVFES
301    ILTGSFPLMS HPSRRGFQAR LSRLHGMSSK NIKKSGGTPT SMVPIPGFVG
351    NKRVMHFSRD SHHDHWHWFSR PAERTEHDHQ LLRQDNRAERG HKKNCVRTA
401    SRQTSMLHGS LTCGDIKRRA KAAPPGLPTT AGFVGENAQP ILENNIGNRM
451    LQMGWTPGS GLGRDGKGIS EPIQAMQRPK GLGLGFPLPK STSATTTPNA
501    GKSA

```

TABLE 12B

HLA peptide motif search results

User Parameters and Scoring Information	
method selected to limit number of results	explicit number
number of results requested	30
HLA molecule type selected	B7
length selected for subsequences to be scored	9
echoing mode selected for input sequence	Y
echoing format	numbered lines
length of user's input peptide sequence	504
number of subsequence scores calculated	496
number of top-scoring subsequences reported back in scoring output table	30

TABLE 3A

Scoring Results

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	400	ASRQTSML	120.000
2	56	HFWETGHCL	80.000
3	476	MQRPKGLGL	40.000
4	29	HSRSITSCPL	40.000
5	474	QAMQRPKGL	36.000
6	4	LVHDLVSAL	20.000
7	139	KVKRMAVDL	20.000
8	100	AKRRPSSNL	18.000
9	423	AFLPGPTTA	6.000
10	454	MGWTPGSGL	6.000
11	396	SVRTASRQT	5.000
12	196	GPKIQDEGV	4.000
13	182	KNKVKKRKL	4.000
14	110	NNVRGKRPL	4.000
15	198	KIQDEGVVL	4.000
16	403	QTSMHLGSL	4.000
17	238	SLSSTDAGL	4.000
18	285	DPTELDKNV	4.000
19	300	SILTGSFPL	4.000
20	347	GPVGNKRMV	4.000
21	480	KGLGLGFPL	4.000
22	417	KRRRAAAMPL	4.000
23	443	ENNIGNRML	4.000
24	313	SRRGFQARL	4.000
25	18	QARGGFAT	3.000
26	293	VPDPVFESTI	2.400
27	295	DPVVFESILT	2.000
28	311	HPSRRGFQA	2.000
29	433	FVGENAQPI	2.000
30	486	EPLPKSTSA	2.000

Echoed User Peptide Sequence
(length = 504 residues)

1 MEELVHDILVS ALEESSEQAR GGFAETGDHS RSISCPLKRQ ARKRRGRKRR
 51 SYNVHHIPWET GHCLSEGSOS SLEEPSKDYL ENHHNNKKDH SDSDDQMLVA
 101 KRPSSNLNN NVRGKRPLWH ESDFAVDNVG NRTLRRRKV KRMADVLPQD
 151 ISNKRTMTQP PEGCRDQDM SDRAYQYQEF TKNVKKKRL KIIROQQPKIQ
 201 DEGVVLESEE TQNQTNKDKME CEEQKVSDEL MSEDSSSSLSTDAGLFTND
 251 EGRRQGDEQS DWFYEKESGG AGCITGVPPW WEKEDPTTELD KNVPDPVFES
 301 ILTGSFPLMS HPSRRGFQA LSRLHGMSK NIKKSGGPT SMVPIPVGPG
 351 NKRMVHFSPD SHHHDHWFSP GARTEHDHQH LLRDNRRAERG HKKNCSVRTA
 401 SRQTSMLHGS LCTGDIKRRR KAAPPLEPPTT AGFVGENAQPI ILENNIGNRM
 451 LQNMGWTPGS GLGLRGKGIS EPIQAMQRPK GLGLGFPLPK STSATTTPNA
 501 GKSA

TABLE 13B

HLA peptide motif search results

User Parameters and Scoring Information	
method selected to limit number of results	explicit number
number of results requested	30
HLA molecule type selected	B7
length selected for subsequences to be scored	10
echoing mode selected for input sequence	Y
echoing format	numbered lines
length of user's input peptide sequence	504
number of subsequence scores calculated	495
number of top-scoring subsequences reported back in scoring output table	30

TABLE 14A

Scoring Results

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	318	QARLsRLHGM	30.000
2	293	VPDPFESIL	24.000
3	345	IPGPvGNKRM	20.000
4	433	FVGEvAQPIL	20.000
5	125	AVDNvGNRTL	18.000
6	99	VAKRvPSSNL	18.000
7	399	TASRqTSMHL	12.000
8	475	AMORpKGLGL	12.000
9	453	NMGWvPGSGL	6.000
10	230	LMSEsDSSL	4.000
11	473	IQAMqRPKGL	4.000
12	312	PSRRgFQARL	4.000
13	425	LPGPtTAGEV	4.000
14	103	RPSSnLNNNV	4.000
15	109	NNNvGKRPL	4.000
16	63	CLSEgSDSSL	4.000
17	315	RCFQaRLSRL	4.000
18	237	SSLsSTDAGL	4.000
19	416	IKRRvKAAPL	4.000
20	196	GPKIqDEGVV	4.000
21	299	ESILtGSFPL	4.000
22	402	RQTSmHLGSL	4.000
23	3	ELVHdLVGAL	4.000
24	147	LPQDlSNKRT	2.000
25	116	RPLWHESDFA	2.000
26	278	VFWvEKEPT	2.000
27	488	LPKStSATTT	2.000
28	292	NVPDpVFESI	2.000
29	45	RGRKvRSYNV	2.000
30	486	FPLPKSTSAT	2.000

**Echoed User Peptide Sequence
(length = 504 residues)**

```

1      MEELVHDLVS ALEESSEQAR GGFAETGDHS RSISCPLKRQ ARKRRGRKRR
51     SYNVHHWPET GHCLSESGDS SLEEPSKDYL ENHNNNNKKDH SDSDDDQMLVA
101    KRRPSNSLNv NVRGKRPRLW HESDFAVONVG NRTLRRRRKV KRMADVLPQD
151    ISNKRTMTQP PEGCRDQMDM SDRAYQYQEFT TKNKVKRRKL KIIROQGPKIQ
201    DEGVVLESEE TNQTNKDKME CEEQKVSDEL MSSESDDSSLS STDAGLFTND
251    EGROQGDEEQS DWFYKEKESGG ACGITGVVPP WEKADPTELD KNVPDPVFES
301    ILTSGPFLMS HPSRRGFQAR LSRLHGMSSK NIKKSGGTPT SMVPIPGPVG
351    NKRMVHFSPD SHHRDHWFSP GARTEHDHQ LLRDNRRAERG HKKNCSVRTA
401    SROTSMLHGS LCTGDIKRRR KAAPLPGPTT AGFVGENAQP ILENNIGNRM
451    LQNMGWTPGS GLGRDGKGIS EPIQAMQRPK GLGLGFPLPK SISATTTPNA
501    GKSA

```

TABLE 14B

HLA peptide motif search results

User Parameters and Scoring Information	
method selected to limit number of results	explicit number
number of results requested	30
HLA molecule type selected	B 3501
length selected for subsequences to be scored	9
echoing mode selected for input sequence	Y
echoing format	numbered lines
length of user's input peptide sequence	504
number of subsequence scores calculated	496
number of top-scoring subsequences reported back in scoring output table	30

TABLE 15A

Scoring Results

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	478	RPKGGLGLF	120.000
2	56	HWPETGHCL	40.000
3	116	RPLWHESDF	40.000
4	425	LPGPTTAGF	20.000
5	334	KSGGTPPTSM	20.000
6	400	ASRQTSMHL	15.000
7	29	HRSRISCPL	15.000
8	196	GPKIQDDEGV	12.000
9	285	DPTELDKNV	8.000
10	239	LSSTDAGLF	7.500
11	139	KVKRMAVDL	6.000
12	223	EQKVSDELM	6.000
13	488	LPKSTSATT	6.000
14	198	KIQDEGVVIL	6.000
15	182	KNKVKKRKL	6.000
16	309	MSHPSRRGF	5.000
17	360	DHHHHDHWF	5.000
18	50	RSYNVHHPW	5.000
19	103	RPSSNLNNN	4.000
20	468	CISEPIQAM	4.000
21	347	GPVGKRMV	4.000
22	398	RTASRQTSM	4.000
23	295	DPVFESILT	3.000
24	476	MQRPKGLGL	3.000
25	74	EPSKDYREN	3.000
26	474	QAMQRPKGL	3.000
27	143	MAVDLPQDI	2.400
28	184	KVKKRKLKI	2.400
29	293	VDPVPFESI	2.400
30	90	HSDSDDQML	2.250

Echoed User Peptide Sequence (length = 504 residues)

```

1      MEELVHDLVS ALEESSEQAR GGFAETGDHS RSISCPLKRQ ARKRRGRKRR
51     SVNHVHPET GHCLSEGSDS SLEEPSKDYR ENHNNNNKDH SDSDDQMLVA
101    KRRPFLNNNN NVRGKRPWLH ESDFADVNG NRTLRRRKV KRMADVLPQD
151    ISNKRMTLQQ PEGCRDQMD SDRAYQYQEF TKNVKKKRL KIIQGPKIQ
201    DEGVVLESSEE TNQTNKDKME CEEQKVSDEL MSESDSSSL STDAGLFTND
251    EGROGQDDEQS DWFYKEKSGC ACGITGVVFW WEKEDPTELD KNVPDPVFS
301    ILTGSFPMLS HPSRQGFQAR LSRLHGMSSK NIKKSGGPTT SMVPIPVGPG
351    NKRVNHFSPD SHHHHDHWFSP GARTEHDHQRL LLRDNRAERG HKKNCNSVRTA
401    SRQTSMHLGS LCTCDIKRNR KAAPLGPTT AGFVGENAQP ILENNIGNRNM
451    LQNMGWTFGS GLGRDGKGIS EPIQAMQRPK GLGLGFPLPK STSATTTPNA
501    GKSA

```

TABLE 15B

HLA peptide motif search results

User Parameters and Scoring Information	
method selected to limit number of results	explicit number
number of results requested	30
HLA molecule type selected	B 3501
length selected for subsequences to be scored	10
echoing mode selected for input sequence	Y
echoing format	numbered lines
length of user's input peptide sequence	504
number of subsequence scores calculated	495
number of top-scoring subsequences reported back in scoring output table	30

TABLE 16A

Scoring Results

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	345	I PGPvGNKRM	40.000
2	70	S SLePSKDY	20.000
3	196	GPKIqDEGVV	18.000
4	318	QARLsRLHGM	18.000
5	14	ESSEgARGF	10.000
6	99	VAKRrPSSNL	9.000
7	103	RPSSnLNnnV	8.000
8	116	RPLWhESDFA	6.000
9	488	LPKStSATTT	6.000
10	293	VPDPvFEsIL	6.000
11	299	ESILTGSFPL	5.000
12	237	SSLsSTDAGL	5.000
13	467	KGIsEPIQAM	4.000
14	56	H PWeTGHCLS	4.000
15	147	LPQDlSNKRT	4.000
16	425	LPGPlTAGFV	4.000
17	358	SPDShHHDHW	3.000
18	230	LMSEsDSSL	3.000
19	253	RQGDdEQSDW	3.000
20	399	TASRqTSMHL	3.000
21	184	KVKKrKLKII	2.400
22	445	NIGNrMLQNM	2.000
23	402	RQTsmHLGSL	2.000
24	300	SILTgSFPLM	2.000
25	334	KSGGtPTSMV	2.000
26	433	FVGBnAQPIL	2.000
27	210	ETNQeNKDKM	2.000
28	63	CLSeGSDSSL	2.000
29	486	FPLPkSTSAT	2.000
30	315	RGFQaRLSRL	2.000

**Echoed User Peptide Sequence
(length = 504 residues)**

```

1  MEELVHDLS ALEESSEQAI GGFIAETGDHS RSISCPLKRQ ARKRRGRKRR
51  SYNVHHPWEI GHCLSEGSDS SLEEPSKDY ENHNNNNKKDI SDSDDDQMLVA
101 KRPSSNLNN NVRGKRLPLWH ESOFAVDNVC NRTLRRRRKV KRAMAVDLQPQD
151 ISNKRTMTQP PEGCRDQMD SDRAYQYQEF TKNKVKKRKL KLRQQGPKIQ
201 DEGVVILESEE TNQTNKDKME CEEQKVSDEL MSESDSSSSL STDAGLFTND
251 EGRQGDEQS DWYEKESEGG ACGITGVVPP WEKEDPTED KNVPDPVfES
301 ITGSGFPLMS HPSRRGFQAR LSRLHGMSSK NIKKSGGTPT SMVPIPGPVG
351 IKNRKVHFSPD SHHHHDHWESP GARTEHDHQO LLRDNRRAERG HKKNCVRTA
401 SRQTSMHLGS LCTGDIKRRR KAALPQGTT AGFVGENAQP ILENNIGNRM
451 LQNMGWTPGS GLGRDGKGIS EPIQAMQRPK GLGLGFPLPK STSATTTPNA
501 GKSa

```

TABLE 16B